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Luther Earl Bishop (1943-1991)

Earl Bishop was born in Orange, California, on 21 October 1943. He was the son of Earl Luther Bishop and Jewel Evelyn Remagen Bishop (later Starkey). Earl's father, an enlisted man in the Army Air Force, was killed in action in World War II when Earl was about a year old. After his father's death, Earl and his mother moved to Nashville, Tennessee. Earl attended the Demonstration High School of the George Peabody College for Teachers, and so had a rigorous education. He joined the American Fern Society in 1959 and attended the Cumberland National Forest foray in August, 1961. The Foray leader, Dr. Donald G. Huttleston, remarked (Amer. Fern J. 51:193. 1961) that "Special mention should be made of Earl Bishop who, although a 1961 high school graduate, was more familiar with the native ferns of the area than were most of the participants, including this reporter."

Earl received a B.S. in Botany and Music *summa cum laude* from the University of Wisconsin, Madison in 1965. In 1967, he participated in a course on tropical ferns conducted in Costa Rica by the Organization for Tropical Studies. He earned a Ph.D. in Botany from the University of Hawaii, Honolulu in 1970, for which he wrote a dissertation on *Adenophorus* (later published in Brittonia 26:217–240. 1974) under the celebrated and demanding Prof. Albert C. Smith. During his Hawaii years, Earl made extensive field trips to five of the islands, discovering several new species and rediscovering several thought to be extinct. While in Alaska, he collected plants (especially *Carex*) and insects (for the Bishop Museum) on the North Slope. As opportunity arose, Earl went on several collecting trips that included the countries of Venezuela, Colombia, Ecuador, Peru (mostly Depto. Chachapoyas), Bolivia, and central Brazil. After receiving his Ph.D., Earl was employed by the University of Hawaii to teach an introductory laboratory course and then by the Honolulu Botanic Gardens to survey, document, and record all the plants growing in the garden. The results of his survey were published by the Garden in 1972 and 1973. After 1973, he resided in Anchorage, Alaska, Seattle, Washington, Berkeley, California, and finally Marquette, Michigan, where he died at the age of 48 from the effects of AIDS on 19 December 1991.

From an early age, Earl was a skilled bird-watcher and bird-bander. He held a U. S. Fish & Wildlife master bird-banding permit and banded birds in Tennessee, Wisconsin, and Alaska. He also was interested in astronomy. He was an avid musician, an excellent organist, and a member of the Seattle Gay Men's Chorus. He was deeply interested in gardening and cultivated many species of lilies *(Lilium)* and orchids *(Dracula* and *Masdevallia).* Because of his proficiency in horticulture, he became a judge of orchids in flower shows, although his color blindedness required him to use a special filter to judge flower color correctly. His descriptions of new genera and species reveal that he was an excellent Latinist with a keen eye for taxonomically significant detail. His taxonomic specialty was the Grammitidaceae, which he was reorganizing into natural genera based on the close observation of morphological and anatomical characters. From 1977 through 1991 he published a number of papers on the genera of Grammitidaceae in the American Fern Journal (67:101–106; 68:76–94; 78:1–5; 79:103–118) and one in Systematic Botany (17:348–362. 1992). This monographic work is being continued by Robbin C. Moran and/or Alan R. Smith (Amer. Fern J. 81:76–88; Novon 2:426 – 432. 1992), and one

papers to follow. His field notebooks and the principal set of his fern collections, mostly Grammitidaceae, are deposited in the herbarium of the University of California, Berkeley.

Earl is commemorated by the genera *Bishopalea* H. Robinson, *Bishopanthus* H. Robinson, *Bishopiella* R. M. King & H. Robinson, *Bishovia* R. M. King & H. Robinson, and *Episcothamnus* H. Robinson (all Compositae), and by the following species (all Compositae, except as noted): *Ageratina bishopii* R. M. King & H. Robinson, *Aspilia bishoplecta* H. Robinson, *Barbacenia bishopii* L. B. Smith (Velloziaceae), *Calea bishopii* H. Robinson, *Cronquistanthus bishopii* R. M. King & H. Robinson, *Dimerostemma bishopii* H. Robinson, *Grammitis bishopii* Stolze (Grammitidaceae), *Grazielia bishopii* R. M. King & H. Robinson, *Helianthopsis bishopii* H. Robinson, *Lychnophora bishopii* H. Robinson, *Mikania bishopii* R. M. King & H. Robinson, *Oyedaea episcopale* H. Robinson, *Perymenium bishopii* H. Robinson, *Trichogonia bishopii* R. M. King & H. Robinson, *Vernonia bishopii* H. Robinson, *Viguiera bishopii* H. Robinson, *Vittetia bishopii* R. M. King & H. Robinson, *Wedelia bishopii* H. Robinson, and *W. episcopalis* H. Robinson — David B. Lellinger, Dept. of Botany NHB-166, Smithsonian Institution, Washington, DC 20560.

Revision of the Genus *Christensenia*

CRISTINA ROLLERI
Laboratory of Botany, Museo de La Plata, Paseo del Bosque, 1900 La Plata, Argentina

The present revision of *Christensenia* is the first for this genus. Observations of external and internal foliar morphology, with particular emphasis on valid taxonomic characters of pinna indument and epidermis and soral paraphyses have shown that the taxa of the genus are *C. aesculifolia* Blume subsp. *aesculifolia* and subsp. *korthalsii* (de Vriese) Rolleri and *C. lobbiana* (de Vriese) Rolleri. Synangium outline, number of sporangia per synangium, and spore morphology are not systematically important. The morphology and physiology of adult porocyclocytic stomates and the presence, chemical composition, and density of microprojections of the mesophyll cell walls are adaptive and vary according to ambient humidity.

The generic name *Christensenia* was proposed by Maxon (1905) to replace the later homonym *Kaulfussia* Blume. De Vriese and Harting (1853) recognized four species of *Kaulfussia, K. aesculifolia* Blume (Java and the Philippines), *K. assamica* Griff. (Assam), *K. korthalsii* de Vriese (Sumatra and the Philippines), and *K. lobbiana* de Vriese (Java). Baker (1874, p. 444) synonymized the three later species in *K. aesculifolia,* and most later scholars agreed, including Maxon (1905), although he did acknowledge variation within the inclusive species *C. aesculifolia* (Blume) Maxon. Christ (1907) described *C. cumingiana* from the Philippines. Christensen (1938, p. 528) mentioned "2 Malayan species" without naming them. Copeland (1909) recognized *C. aesculifolia,* distributed from Malaysia to northern India, and *C. cumingiana,* from the Philippines. Later, Copeland (1958, pp. 27–28) reduced the latter species to *C. aesculifolia* and stated for it a general distribution from India to the Philippines, including Sumatra and Java. A similar concept was adopted by Holttum (1955, pp. 45–46). Ching (1940) separated *Christensenia* from the Marattiaceae into a new family Christenseniaceae and recognized two species, *C. aesculifolia* and *C. cumingiana,* to which he later (Ching, 1959, p. 65) added *C. assamica* (Griff.) Ching. Pichi Sermolli (1970, p. 234) adopted Ching's segregation of the Christenseniaceae. Braithwaite (1977), Walker (1979), Hill and Camus (1986), and Camus (1990, p. 177–178) analyzed different aspects of the genus, but considered it to be monospecific.

MATERIALS AND METHODS

Specimens were examined from the Muséum National d'Histoire Naturelle, Paris (P), the Instituto Darwinion, San Isidro (SI), and the United States National Herbarium, Washington (US). Some 50–80 microscope slides were made of fragments from each taxon and are deposited at the Museo de La Plata.

Adult pinnae were cleared for 24 hr. with aqueous 2.5% NaOH and saturated aqueous chloral hydrate. To minimize fragility of the samples after they were cleared, they were treated with a mixture of 540 ml 95% ethanol, 210 ml distilled water, 240 ml glycerine, and a few drops of formalin, which gave them elasticity and new strength. Staining material after this treatment gave better results.

General stains used were ferric chloride-tannic acid (Foster, 1934), Chlorazol Black E (Gurr, 1966, p. 275), Safranin-Aniline Blue (Gurr, 1966, p. 306), and 1% aqueous Ruthenium Red. Several authors (Johansen, 1940, p. 200; Venning, 1934, p. 20, and

D'Ambrogio de Argüeso, 1986, p. 76) used different concentrations of Ruthenium Red; I found that 1% is adequate and fast-acting (20–30 sec.).

Specific stains used to reveal the nature of epidermal cell walls, papillae and trichome walls, and microprojections of mesophyll cell walls were Toluidine Blue and zinc chloride-iodine (D'Ambrogio de Argüeso, 1986, pp. 70, 72), Indophenol Blue (Peacock, 1966), Methylene Blue (Johansen, 1940, pp. 200), and Ruthenium Red, as explained above.

Specific stains and methods used to reveal the nature and contents of trichome cells were the preceding (except for zinc chloride-iodine) plus Lugol's Solution, iodine (both according to Johansen, 1940, pp. 183, 189 and D'Ambrogio de Argüeso, 1986, p. 75), Sudan IV in ethylene glycol (Gurr, 1966, p. 313–314), the Per-iodic-Schiff (PAS) method (Gurr, 1966, p. 296–298), and Sudan IV in saturated isopropanol, a technique I developed.

Epidermal Patterns and Cell Walls

Cell sizes and wall patterns differ in the epiphyll and hypophyll. Wall patterns seen in cleared preparations are straight, angular, or sinuous (Figs. 1L, H, E), and the latter two kinds may be either frequent or subfrequent (e.g., sinuous-frequent, angular-subfrequent). Walls themselves seen in cross-section show different degrees of thickening across the walls and differences in regularity of thickening along the walls.

The epiphyll cells of *C. aesculifolia* subsp. *aesculifolia* (Fig. 1A) are larger and have slightly thinner walls (Fig. 1B) than those of the other taxa (Figs. 1E, F, I, J). The cells are elongate (l:w = 2–3:1) in *C. aesculifolia,* but are nearly isodiametric in *C. lobbiana.* All epiphylls have a sinuous-frequent pattern (Figs. 1A, E, I). The sinuosities are slightly more marked in *C. lobbiana* (Figs. 1I, J) than in the other taxa. The wall thickenings are generally more marked in *C. aesculifolia* subsp. *aesculifolia* and in *C. lobbiana* (Figs. 1B, J) than in some material of *C. aesculifolia* subsp. *korthalsii* (Fig. 1F), and the latter subspecies has more irregular thickenings.

The epiphyll patterns of *Christensenia* are noticeably like those of some *Danaea* species, especially *D. nodosa* (L.) J. E. Smith, whereas *Marattia* and other *Danaea* species have angular epiphylls (Rolleri et al., 1987).

The hypophylls are more variable than the epiphylls. In *C. aesculifolia* subsp. *aesculifolia,* the pattern is sinuous-subfrequent with shallower sinuosities, the cells are somewhat elongate (1:w = 2–3:1), and the cells are more irregularly thickened than in the epiphyll (Figs. 1C, D). In *C. aesculifolia* subsp. *korthalsii,* the pattern is angular-subfrequent, the cells are more elongate (1:w = 2–4:1), and the thickened areas of the cells are irregular (Figs. 1G, H). In *C. lobbiana,* the pattern is polygonal, some of the cells are subisodiametric (1:w = 1–2:1), and the walls are irregularly thickened (Fig. 1K, L).

Stomata

The stomata are basically cyclocytic. The guard cells are strongly arcuate and circumscribe the stomatal pore, which may be up to 200–240 μm in diam. and which may be found in every degree of openness, presumably depending upon ambient climate. The subsidiary cells are arranged in 2–3(5) cycles of narrow, arcuate cells that are distinct from the adjacent epidermal cells. Unlike most stomata, all the cells in *Christensenia,* including even the fourth and fifth cycles of subsidiary cells, protrude above the plane of the epidermis.

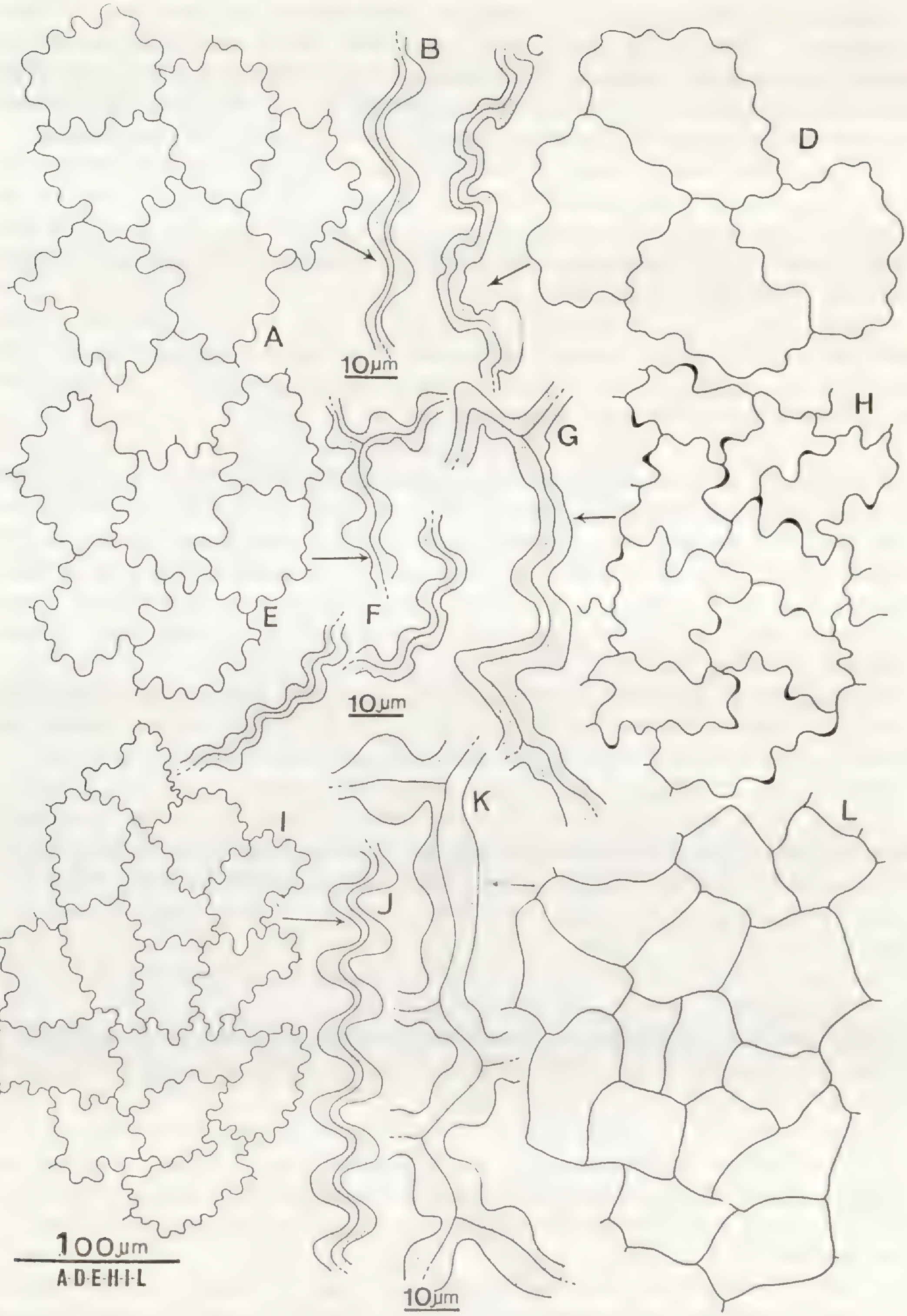

Fig. 1. Epidermal patterns and details of cell walls in *Christensenia*. A–D. *C. aesculifolia* subsp. *aesculifolia*. A, B. Epiphyll. C. D. Hypophyll. E–H. *C. aesculifolia* subsp. *korthalsii*. E, F. Epiphyll. G, H. Hypophyll. I–L. *C. lobbiana*. I, J. Epiphyll. K, L. Hypophyll.

Rolleri et al. (1991a) studied the ontogeny, development, and physiology of these stomata in *C. lobbiana* (as *cumingiana),* which they named porocyclocytic. In this species the ontogeny is perigenous. The typical cyclocytic stomata of other genera of the Marattiales, which also can be seen in apical and marginal areas of *Christensenia* pinnae, represent a phase toward the fully developed porocyclocytic stomata of *Christensenia.*

In *C. aesculifolia* subsp. *aesculifolia,* the paired guard cells are very large and strongly arcuate; in subsp. *korthalsii* a third guard cell has rarely been seen; in *C. lobbiana,* the guard cells occasionally divide anticlinally after the pore is formed, and up to 5 of them can be found in a single stoma in the pinna apices, intermingled with more typical stomata having a single pair of guard cells.

Rolleri et al. (1991a) interpreted the porocyclocytic stomata as an adaptation to the functions primarily of gas exchange and secondarily of liquid water secretion, as if the stomata were hydathodes. Given the highly humid ambient conditions of *Christensenia,* the stomata probably act in these ways alternately.

TRICHOMES

The epiphylls of *Christensenia* are always glabrous. The hypophyll of *C. aesculifolia* subsp. *aesculifolia* is subglabrous (Figs. 2A–E) to glabrous, with a scattering of branched hairs and a few uniseriate hairs. The costae and veins bear a few scales and occasionally smooth papillae. The hypophylls of the other taxa have a random mixture of indument types in the veins and lamina surface, giving them a characteristic trichomatose appearance that is easily seen at low magnifications. The hypophylls of *Danaea* bear a similar indument (Rolleri et al., 1991b).

The trichomes of *Christensenia* are all glandular in nature. Tests for fats were strongly positive for capitate cells and papillae, whereas tests for starch, saponine, essential oils, inuline, and proteins were negative. In contrast, the trichomes of *Danaea* are tanniferous. Trichome walls are cellulosic with weak traces of a cutin layer. Although four kinds of trichomes can be distinguished, intermediate stages between papillae and unbranched hairs, between unbranched and branched hairs, and between branched hairs and scales are known, and all probably share a common ontogenetic ancestor. This is confirmed by microchemical assays, which tend to show that all trichomes of the hypophyll are of the same origin.

Globose papillae. — Protruding, dome-shaped, reddish-brown papillae with a smooth or irregular surface (Figs. 3A, Y, A', F', 4A–B).

Uniseriate hairs. — Straight or curved, with a cylindrical or subconical basal cell, 1 to 7 cylindrical body cells that can be shorter than the basal cell, and a capitate apical cell (Figs. 2F–J, A'–C', 3B–E, I–N, B'–F').

Branched hairs. — Straight or rarely curved, with a cylindrical or subconical basal cell, a paucicellular body prolonged into short, paucicellular or uniserjate processes, and a capitate cell at the apex of each process. These trichomes are very diverse (Figs. 2A–E, S–Y, D', 3F–G, P–U). Some appear to be transitional between uniseriate hairs and branched hairs (Figs. 2K, 3O).

Scales. — Pluricellular, with a cylindrical basal cell, a partially to totally flattened body, with very short, paucicellular or unicellular processes, and a capitate cell at the apex of each process (Figs. 2Z, E', 3H, V–X, G'). Some appear to be transitional between branched hairs and scales (Figs. 2L–R, 3P–T). Despite their flattened bodies, these scales are not coordinate with the scales that are typical of the higher ferns, which appear to dif-

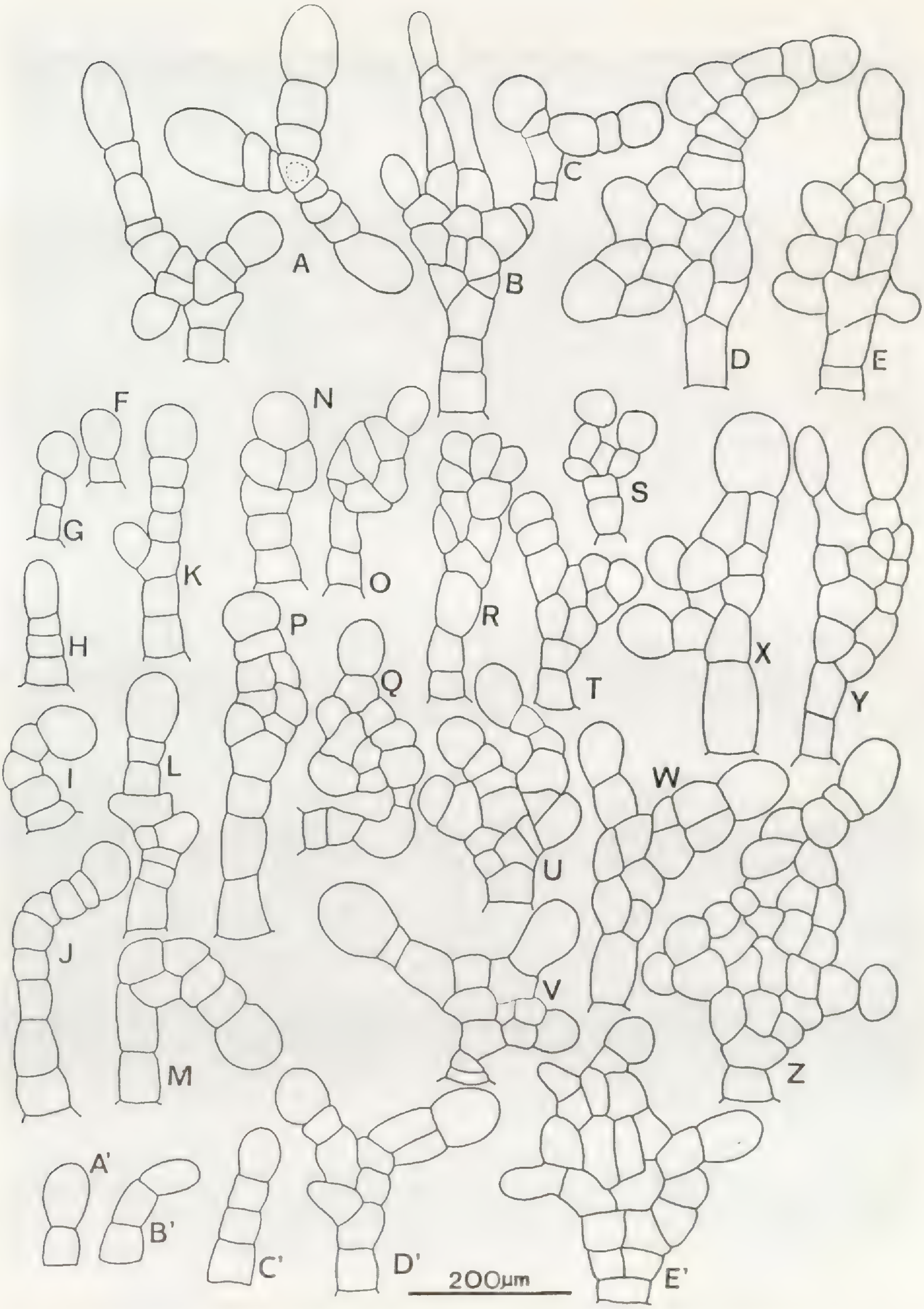

Fig. 2. Trichomes of the lamina surface in *Christensenia*. A–E. *C. aesculifolia* subsp. *aesculifolia*. F–Z. *C. aesculifolia* subsp. *korthalsii* and *C. lobbiana* types in common. A'–E'. Additional types in *C. lobbiana* (superficial papillae not shown).

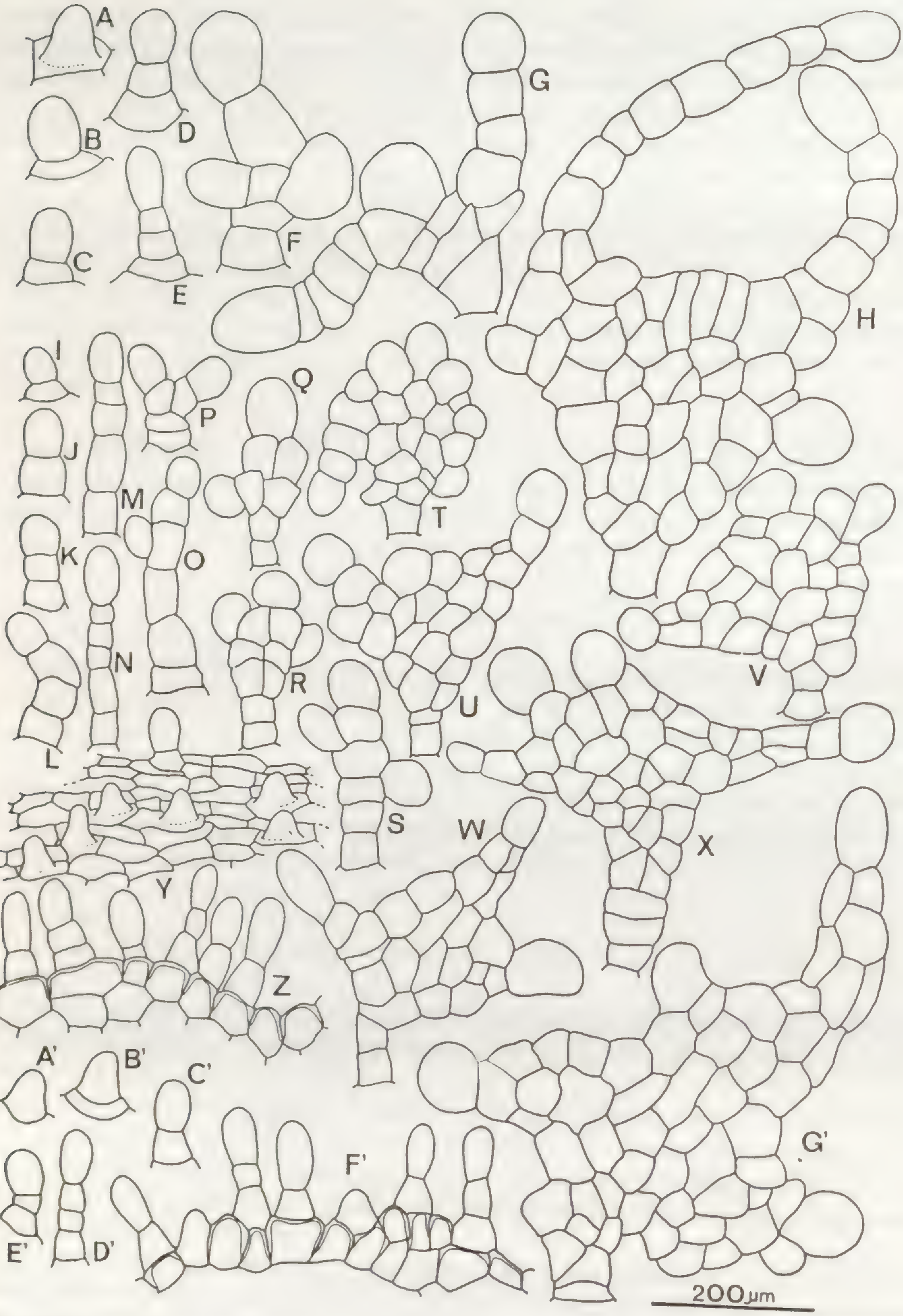

Fig. 3. Trichomes of the veins in *Christensenia*. A–H. *C. aesculifolia* subsp. *aesculifolia*. I–Z. *C. aesculifolia* subsp. *korthalsii*. A'–G'. *C. lobbiana*. Y. Secondary vein as seen in surface in *C. aesculifolia* subsp. *korthalsii*. Z. Same in cross-section. F'. Cross-section of vein. H, X, and G. Scales.

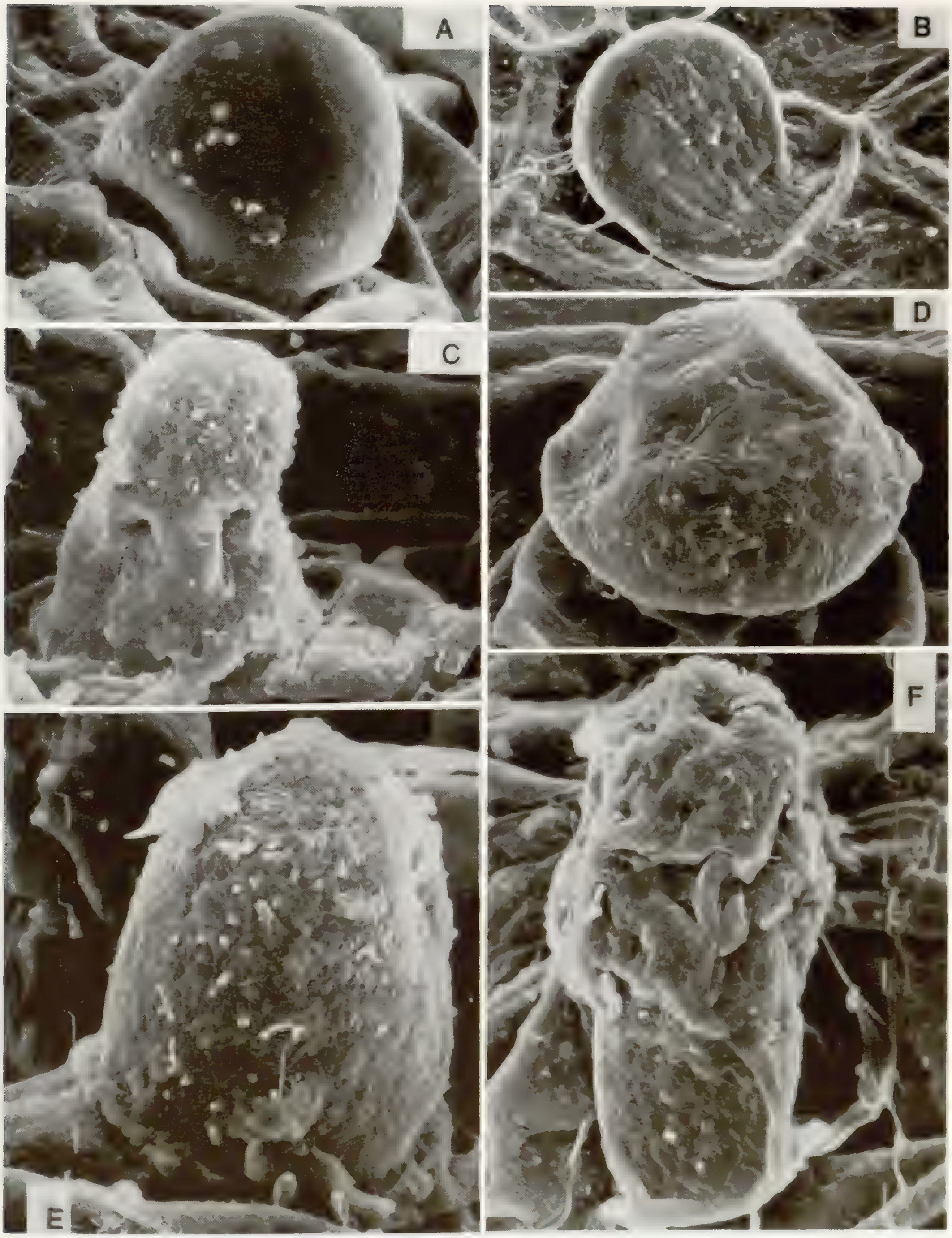

Fig. 4. SEM photographs of trichomes in *Christensenia*. A. Smooth papilla on secondary veins of *C. aesculifolia* subsp. *korthalsii* (abundant and dense). B. Small, superficial papilla in *C. lobbiana*. C-D. Bicellular trichomes in *C. aesculifolia* subsp. *korthalsii*. E. Large, rugose papilla on lamina and near veins in *C. lobbiana*. F. Bicellular trichome in *C. lobbiana*. Magnifications: A-B, x1500; C-F, x2000.

fer more markedly from hairs. Although they are the final developmental phase of trichomes in *Christensenia,* they appear to be an expansion of branched hairs.

Microprojections of Mesophyll Cell Walls

The laminae of *Christensenia* lack a typical photosynthetic palisade layer. The mesophyll cells are uniform, Y-branched, and connected by their ends, leaving wide intercellular spaces. They can be seen clearly through the ample stomatal pores and their most outstanding characteristics, the rod- or club-shaped microprojections of their outer walls (Figs. 5, 6). Luerssen (1873) illustrated them for *C. aesculifolia* sensu lato (as *Kaulfussia)* and also noted their appearance in roots, rhizomes, and stipes of *Angiopteris* (Luerssen, 1875). He thought the microprojections were composed of cellulose and cutin. Bary (1884) described them as projections of the cellular walls. Gardiner (1885) described them as composed of mucilage in *Aspidium filix-mas, Blechnum brasiliense,* and other ferns. Schenk (1886) stated that their composition does not include cellulose or waves and that their biological meaning was enigmatic. Campbell (1911, p. 204) described them as "mainly composed of calcium pectate." Pettit (in Hill and Camus, 1986) suggested that they do not contain either proteins or lipids and that tests point to the presence of polysaccharides.

The microchemical tests I carried out indicated a general presence of hydroxylate polysaccharides (predominately cellulose) and traces of pectinate mucilages in the rounded ends of the microprojections and also in their lateral walls (mixed with cellulose).

The microprojections vary in density and relative size. The longest and narrowest microprojections are found in *C. aesculifolia* subsp. *aesculifolia,* especially from the Solomon Islands (Figs. 5D–E); the shortest, sometimes verrucose and with rounded ends, are found in C. *aesculifolia* subsp. *korthalsii* (Figs. 6D). The microprojections of *C. lobbiana* are similar to those of *C. aesculifolia,* but are more densely distributed (Figs. 6E–I). Variability may be related to habitat, rather than being taxonomically diagnostic. One could hypothesize that the presence of microprojections is related to the efficiency of water movement and retention within the pinnae. The mesophyll is poorly developed, only a few layers thick, and so the movement of water and water vapor within it would be strongly facilitated by the large stomatal pores. The microprojections on the outer walls of mesophyll cells would enlarge their hygroscopic surface, insuring a constant and dynamic water balance. The mucilaginous tips of the microprojections suggests this hygroscopic function.

Synangial Receptacles and Paraphyses

The synangia of *Christensenia* are elevated above the lamina surface by parenchymatous receptacles that appear dome-shaped in cross-section. The parenchyma on which the synangium lies is sclerosed, and the cells are lignified (microchemical tests indicate a high concentration of polyphenols in the cell walls). When the synangium is shed, the circular or elliptical scar formed by the dark brown, sclerosed cells is easily visible. Hill and Camus (1986) did not study the receptacles in detail and used the term placenta for them. However, that term has angiospermic connotations and need not be used in preference to the term receptacle.

Paraphyses are borne on the perimeter of the receptacles. Although paraphyses stain intensely with Ruthenium Red, they are eglandular and their cell walls are very thin and

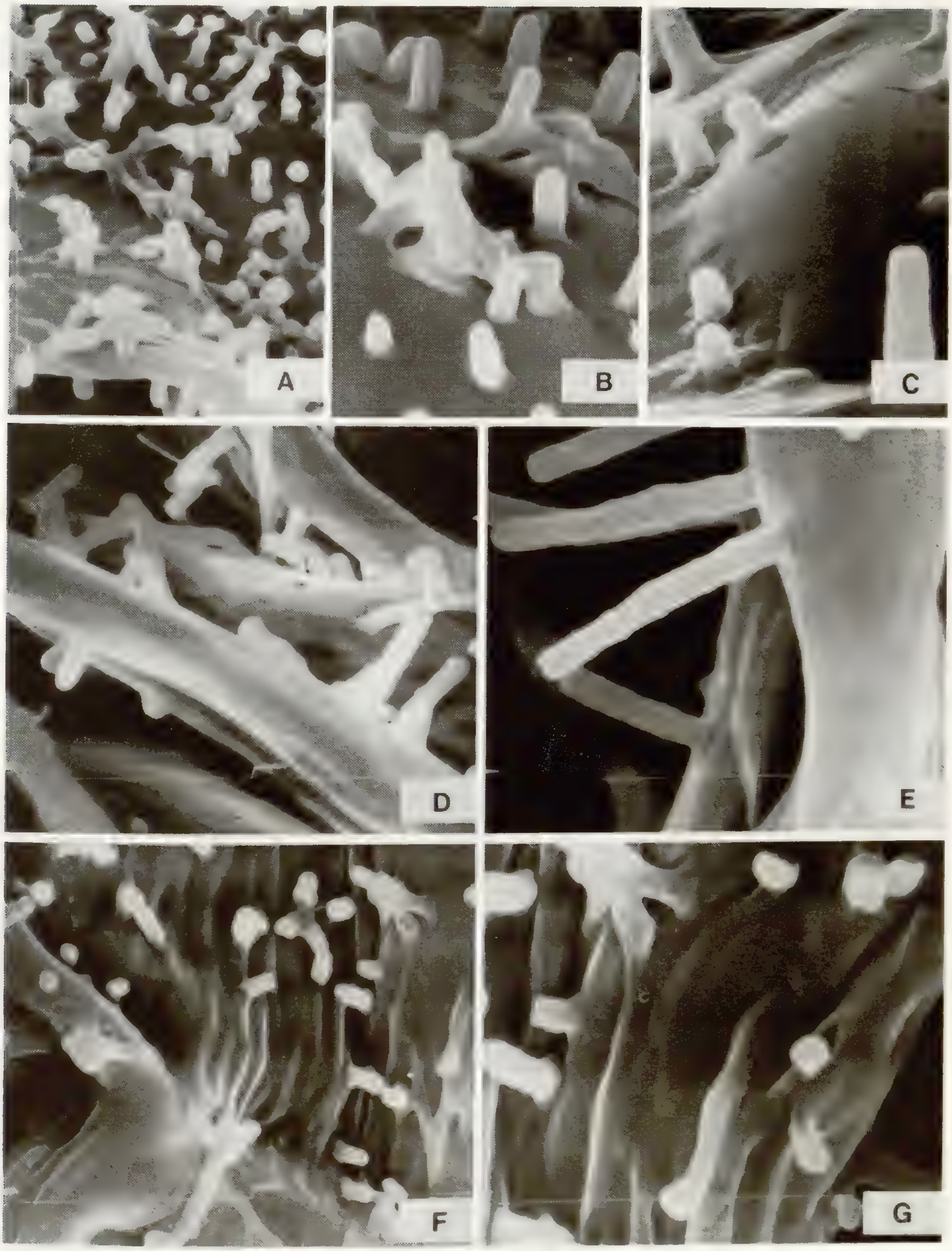

Fig. 5. Microprojections of mesophyll cell walls in *Christensenia aesculifolia* subsp. *aesculifolia*. A–C. From Java (*Zollinger 1902*, P). D–E. From the Solomon Islands (*Braithwaite 4220*, P). F–G. From Java (*Buysman 2607*, P). Magnifications: A, D, F, X5000; B, C, E, G, X10000.

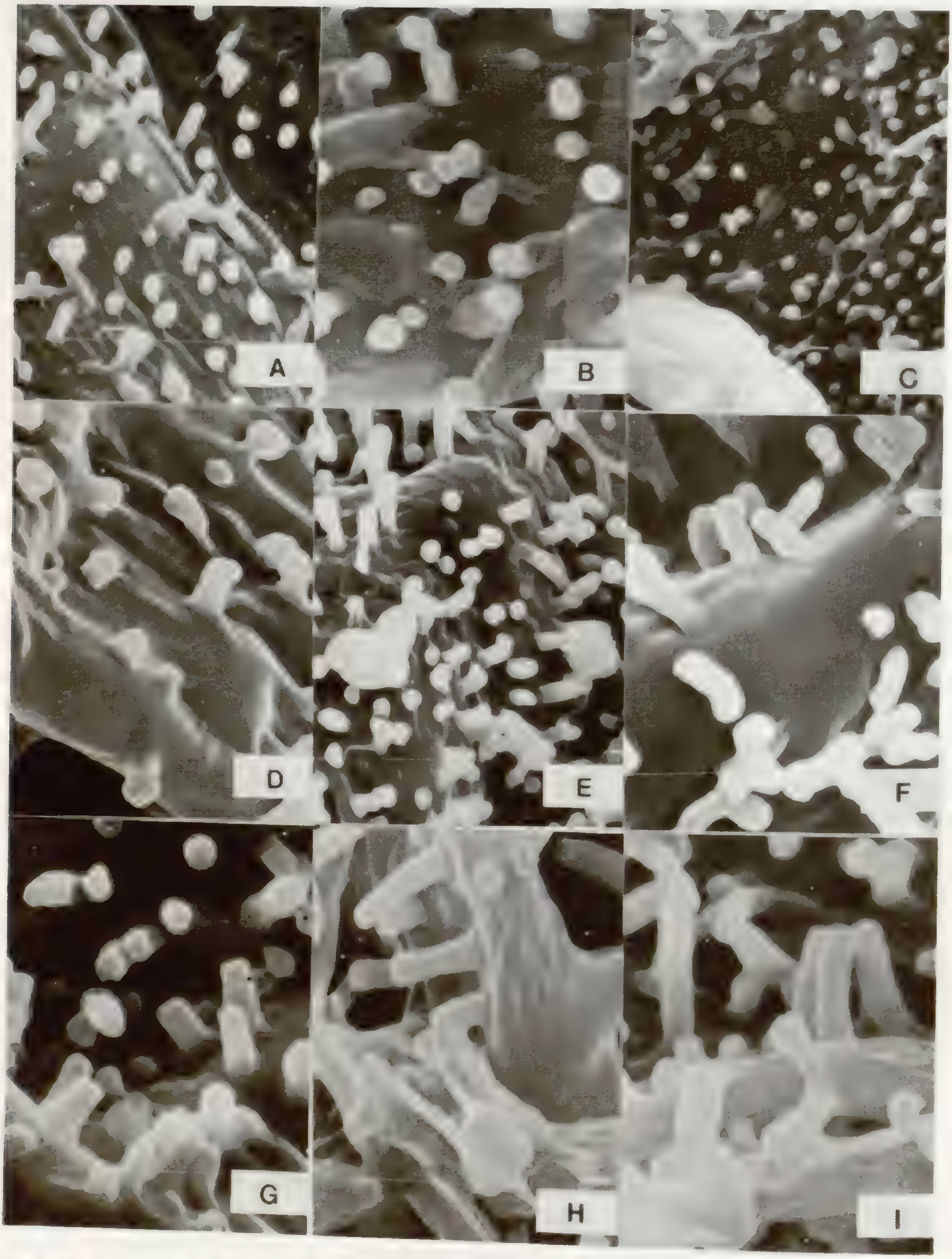

Fig. 6. Microprojections of mesophyll cell walls in *Christensenia*. A–B. *C. aesculifolia* subsp. *korthalsii* from Sumatra (*Brooks 70.S*, P). C–D. Same, from the Philippines (*Copeland 2063*, P). E–I. *C. lobbiana* from the Philippines. E–F. *Copeland s.n.* (P). G–H. *Edaño 36839* (P). I. *Robinson 17914* (P). Magnifications: A, C, E, X5000. B, D, F–I, X10000.

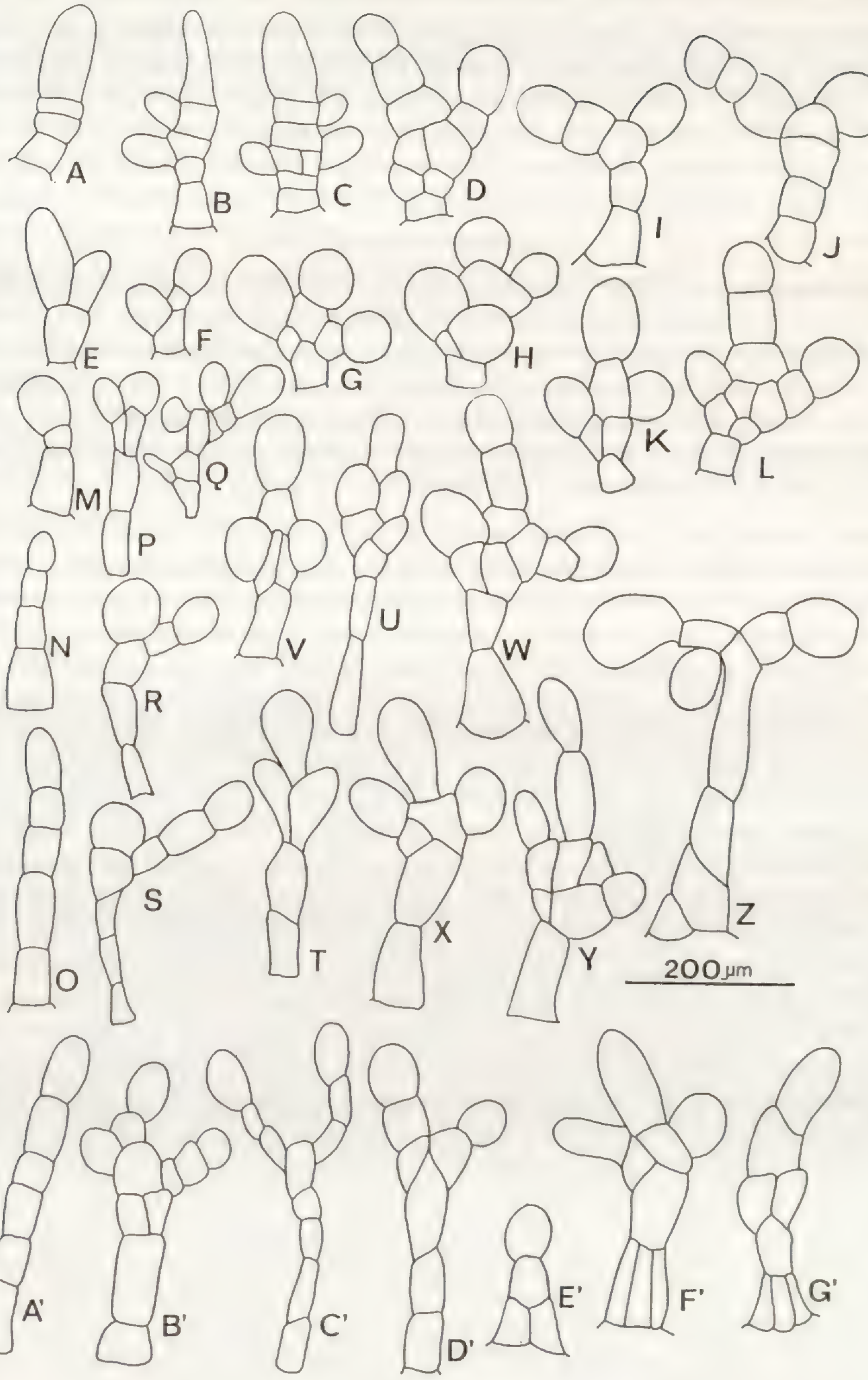

Fig. 7. Paraphyses in *Christensenia*. A–J. *C. aesculifolia* subsp. *aesculifolia*. K–Z. *C. aesculifolia* subsp. *korthalsii*. A'–G'. *C. lobbiana*.

cellulosic. Some of the hair-like paraphyses differ in shape and density in the taxa. Most paraphyses are uniseriate (Figs. 7A, M–O, A'), but a few are Y-shaped (Figs. 7I–J, Z, C'–D'). In *C. lobbiana,* paraphyses with a 3-celled base were seen (Figs. 7F'–G'). A few paraphyses resemble variously branched hairs (Figs. 7W–Y). Paraphyses having the form of small triangular, caducous scales have been observed occasionally at the edge of receptacles of young synangia of all taxa. These are of no taxonomic significance. Hill and Camus' (1986) term placental hair need not supplant the more usual term paraphysis.

Taxonomic Treatment

Christensenia Maxon, Proc. Biol. Soc. Wash. 18:239. 1905. — Type: A renaming of *Kaulfussia* Blume, and so based on the type of that name.

Kaulfussia Blume, Enum. Pl. Javae 2:260. 1828, nec Dennst. Schlüssel Hortus Malab. 30. 1818, nec Nees Horae Phys. Berol. 53, f. 11. 1820. – Type: *Aspidium aesculifolium* Blume [= *Kaulfussia aesculifolia* (Blume) Blume].

Macrostoma Griff. Asiat. Res. 19:108. 1836, non Hedwig, Gen. Pl. 102. 1806, which is *Macrostema* Pers. Syn. Pl. 1:185. 1805.

Plants terrestrial, at 1–1000 m elevation, usually in very dark shade in undisturbed forests on banks of small streams or rivers or on humid slopes. Rhizomes short-creeping, subascending, or erect, carnose to subcarnose, scaly, the scales basifixed, wide and rounded with undulate margins, castaneous with reddish spots. Stipes 1 or more, 8–35(45) cm long, stiff, carnose, slightly to deeply canaliculate, papillose and with scattered trichomes, the papillae very dense, bright reddish-brown, the trichomes similar to those of the costae. Fronds simple, ternate, or palmate with five pinnae. Laminae lanceolate or broadly so in simple fronds, nearly symmetrical to irregular, usually with a shallowly cordate or irregularly rounded base, this sometimes widened and undulate, with an acute, acuminate, or rarely obtuse-cuspidate apex. Pinnae of compound fronds sessile or nearly so, in ternate laminae the terminal one generally symmetrical, with a cuneate base and an acute or acuminate apex, the lateral pair pedate, sometimes quite reduced, in palmate laminae the terminal pinna similar, the basal pair strongly pedate or reduced or even curved. Laminae herbaceous, spongy, subcoriaceous, coriaceous, or carnose, the epiphyll smooth, bright dark green, the hypophyll opaque, light green to pale yellow. Costae and primary veins abaxially rigid and prominulous, roughly striate, indurated, and subglabrous, or slightly prominulous, not indurated, and distinctively papillose-trichomatose. Secondary veins anastomosing to form a reticulum of polygonal areolae with free included veinlets. Trichomes, if present, all glandular, a mixture of papillae, uniseriate hairs, branched hairs with a paucicellular body, and more elaborated scales with a pluricellular body and uniseriate processes, all with thin, cellulosic, slightly cutinized walls. Synangia circular to elliptic, the fused sporangia 8–12. Receptacles prominulous, mostly parenchymatous, but with the surface cells sclerosed. Paraphyses bordering the receptacles uniseriate and pluricellular or more commonly similar to the branched hairs of the lamina, but with more delicate, thinner walls and lacking glandular apical cells. Spores monolete, elliptic in polar view, with a conical/spinulose ornamentation of close, short, sharp, but soft, spine-like processes throughout.

Distribution: India, Burma, Malaya, Sumatra, Java, Borneo, the Philippines, and the Bismark and Solomon Islands (Fig. 8).

KEY TO CHRISTENSENIA

1. Plants small to medium (up to 35 cm long); stipes densely papillose, often carnose, (9)10–23(25) cm long; fronds simple, occasionally ternate, rarely palmate, the lamina (or terminal pinna in compound fronds) lanceolate to broadly so, 9–10(15) cm long, 3–4(9) cm wide; lateral basal pinnae, if any, reduced in ternate fronds, pedate in palmate ones; laminae herbaceous to spongiose, often subcarnose; veins and lamina surface papillose-trichomatose, the trichomes diverse, bright, reddish-brown throughout. Laminae green adaxially, yellowish-green abaxially.....3. **C. lobbiana**

1. Plants medium to large (30–80 cm long); stipes (6)18–36(50) cm long, not densely papillose, stiff, deeply canaliculate in large fronds; fronds palmate, occasionally ternate, rarely simple, the terminal pinna (or lamina in simple fronds) lanceolate to rhomboidal, 15–18(25) cm long, 7–9(15) cm wide; lateral suprabasal pinnae, if any, asymmetric to pedate; lateral basal pinnae, if any, pedate; laminae herbaceous, papyraceous, subcoriaceous, or coriaceous; veins and lamina surface papillose-trichomatose, subglabrous, or glabrous, the trichomes, if any, diverse or reduced, yellowish except for the reddish-brown glandular cells.....2

2. Indument diverse, the costae, veins, and lamina surface mostly papillose-trichomatose, the last abundantly so, the trichomes reddish-brown to reddish, mixed with dense papillae; laminae herbaceous to papyraceous, dark green adaxially, pale green abaxially.....
2. **C. aesculifolia** subsp. **korthalsii**

2. Indument reduced to absent, the veins with scattered scales and occasional papillae not visible to the naked eye, the lamina surface with a few trichomes but no papillae; laminae subcoriaceous to coriaceous, green adaxially, pale green abaxially (or slightly yellowish in specimens from the Bismarck Archipelago and the Solomon Islands.....
1. **C. aesculifolia** subsp. **aesculifolia**

1. **Christensenia aesculifolia** (Blume) Maxon, Proc. Biol. Soc. Wash. 18:240. 1905, subsp. **aesculifolia.** — *Aspidium aesculifolium* Blume, Enum. Pl. Javae 2:143. 1828. — *Kaulfussia aesculifolia* (Blume) Blume, Enum. Pl. Javae 2:260. 1828. — TYPE: Java, "crescit in Javae Provinciae Bantam," *Blume* (holotype L not seen Morton photo 766 US; isotype P? not seen).

Plants terrestrial, robust, up to 70(80) cm long; rhizomes erect to suberect, short, carnose, scaly, the scales large, rounded, undulate to subentire, bicolorous, castaneous with reddish spots; stipes fleshy when young, stiff and canaliculate at maturity, subpapillose and with scattered, small, castaneous-reddish scales; fronds palmate or occasionally ternate, the terminal pinna rhomboidal to broadly lanceolate, up to 22–25 cm long, 11– 15 cm wide, cuneate at the base, acute to acuminate at the apex, the distal pinnae, if any, asymmetrical, the proximal pinnae pedate to almost crescent-shaped; laminae papyraceous to coriaceous, their margins entire or slightly undulate, revolute, subpapillose; costae prominent, rigid, indurated, glabrous to subglabrous, with scattered, variously branched hairs and scales; primary veins prominulous and rigid; lamina surface glabrous to subglabrous, sometimes with scattered, variously branched hairs, papillae absent; synangia usually circular, the fused sporangia 8–10; receptacles circular to elliptical;

Fig. 8. Geographical distribution of the taxa of *Christensenia*. Solid line = *C. aesculifolia* subsp. *aesculifolia*. Dotted line = *C. aesculifolia* subsp. *korthalsii*. Dashed line = *C. lobbiana*.

paraphyses commonly short, paucicellular, simple, forked, or branched; scales small, caducous.

Cytology. — Previous cytological studies recorded $2n = 80$ for plants from Borneo (Walker, 1979) and $2n = 160$ for plants from the Solomon Islands (Braithwaite, 1977). Both numbers are based on $x = 40$, also known for other genera of Marattiales. Although the Solomon Island plants are tetraploid, they show no essential morphological differences from other, presumably diploid, specimens of this subspecies. At present, it seems best to include the tetraploids in subsp. *aesculifolia* pending further study, especially of the nature and origin of their tetraploid condition.

Specimens examined: JAVA: Salak, *Raciborski s.n.* (US); Batavi, Perbati, W slope of Mt. Salak, *Palmer & Bryant 670* (US); Basamala, *Raciborski s.n.* (P); Indat [?] Mountain, *Forbes 550* (US); near Bogor, *Buysman 2607* (P); without specific locality, *Zollinger 1902* (P). BISMARCK ARCHIPELAGO: New Ireland: Namatanai, Mandiu Lake 6 km WNW of Taron, *Croft (LAE) 68399* (US). SOLOMON ISLANDS: E. San Cristóbal, lower reaches of Sumaro River, *Braithwaite 4215* (BM, P); E. San Cristóbal, just below confluence of Warahito and Pagato rivers, *Braithwaite 4220* (BM, P).

2. Christensenia aesculifolia subsp. **korthalsii** (deVriese) Rolleri, comb. nov. — *Kaulfussia korthalsii* de Vriese, Ned. Kruidk. Arch. 1(3):186. 1852. — TYPE: Sumatra, *Korthals s.n.* (holotype L not seen Morton photo 767 US).

Kaulfussia assamica Griff. Asiat. Res. 19:108. 1856. — *Christensenia assamica* (Griff.) Ching, Fl. Reipub. Pop. Sinica 2:65. 1959. — TYPE: "Assam," *Griffith 1696* (holotype BM not seen photocopy BM; isotype P).

Plants terrestrial, flexible, delicate, up to 40–45 cm long; rhizomes short, carnose, scaly, the scales adherent, large, castaneous with reddish spots, the margins entire to undulate; stipes subcarnose in smaller plants (juveniles?) to somewhat rigid, stiff and striate in the largest plants, scaly, the scales scattered, small, peltate, castaenous to reddish brown; fronds ternate, occasionally palmate, rarely simple, the terminal pinna (or lamina in simple fronds) lanceolate to narrowly so, 15–18 cm long, 7–9 cm wide, slightly ascending and curved; laminae herbaceous, rarely papyraceous, with plane or slightly revolute margins, these subpapillose, usually undulate, serrate, or serrate-denticulate, rarely entire; costae and veins papillose-trichomatose, densely covered with papillae, the hairs and scales diverse; synangia commonly circular, the sporangia 8–12; receptacles circular; paraphyses similar to branched trichomes, but eglandular and more delicate.

Specimens examined: INDIA: Assam, Margherita, Lakhimpur District, *Mann s.n.* (US); "Assam, Ind. angl.," *Griffith s.n.* (P, possible isotype of *K. assamica*); Assam, Makumi Forest, Lakhimpur, *Mann s.n.* (P). MALAYA: Selangor, *Molesworth-Allen 2439* (US); Perak, Larut, *King 4784* (P); Perak, *Scortechini s.n* (P); Perak, Batang Padang, *Ridley 13927, 13928* (P); without locality, *Ridley 14244* (P). SUMATRA: Lebong Tandai, Benkoelen, *Brooks 70.S* (P, US), *113.S* (P); without locality, *Hancock 29* (US); Sarawak, near Kuching, above 'Rajah' bungalow 3/4 of the way to Mt. Matang, *Molesworth-Allen 8146* (US); Central Kalimantan, Bukit Raya and upper Samba River 60–80 km NNW of Tumbang Samba, base camp Tumbang Riang, *Mogea 3637* (US). PHILIPPINES: Mindanao, Davao, Todaya, Mt. Apo, *Elmer 11275* (P, US); Negros, Luzon, *Copeland 2063* (P, US); Camarines, Mt. Isarong, *Ramos 7513* (P, US); Mt. Maquiling, *Copeland s.n.* (P); Luzon, Rizal, Panintingan, *Loher 13568* (P).

3. Christensenia lobbiana (de Vriese) Rolleri, comb. nov. — *Kaulfussia lobbiana* de Vriese in de Vriese and Harting, Monogr. Marattiac. 14. 1853. — TYPE: "Habitat in insulam Javam," *Lobb* (K-Hb. Hook. not seen). [Note the polygonal epidermal pattern of the hypophyll illustrated in figs. 5A—D.]

Christensenia cumingiana Christ, Philippine J. Sci., Bot. 2:186. 1907 — TYPE: Philippines, Mindanao, Prov. Zamboanga, *Copeland s.n.* (MICH not seen; possible isotype US).

Plants terrestrial, generally small, (18)20–30(32) cm long; rhizomes suberect to short-creeping, short, bearing several stipes, scaly, the scales large, round, adherent, castaneous with reddish spots, with undulate or entire margins; stipes carnose, often slightly striate, not rigid or canaliculate, pale yellow with red spots, papillose; fronds simple or rarely ternate; simple laminae and terminal pinnae of ternate laminae lanceolate; lateral pinnae asymmetrical, pedate, sometimes reduced, sessile, lanceolate to triangular, rounded-lobate, rounded-undulate, and very irregular (small pinnae occasionally asymmetrically cuneate) at the base, the margins undulate, serrate, or lobate, the laminae usually spongiose; reddish to castaneous-reddish, glandular papillae, hairs, and scales of all types present on the costae, veins, and lamina surface, contrasting with the pale yellow hypophyll; synangia frequently elliptic, with 9–12 fused sporangia; receptacles elliptic or circular; paraphyses abundant, similar to branched trichomes, but delicate and eglandular.

Specimens examined: BURMA: Warzup [Warazup], *Sidney 2* (US). MALAYA: Kepong, Sungei Kerod, near Kuala Lumpur, Selangor, *Molesworth-Allen 24344* (US).

SUMATRA: Indragiri, "in silvis prope pl. Lalh.," *Burchard 46* (P). PHILIPPINES: Mindanao, Prov. Zamboanga, San Ramón, *Copeland 152* (US, possible icotype of *C. cumingiana*), *Copeland s.n.* (P, possible isotype of *C. cumingiana*); Mindanao, Zamboanga, *Edaño 36838, 36839* (both P, US); Capiz, Panay, *Ramos & Edaño 31199* (US); Luzon, Mt. Maquiling, *Robinson 17914* (P, US), *Robinson 17199* (US); Sorsogon, Mt. Bulusan, *Elmer 15915* (P, US).

ACKNOWLEDGMENTS

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The relationship of the fossil fern *Schizaeaopsis* Berry to modern genera in the Schizaeaceae

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The fossil fern *Schizaeaopsis* was described by Berry (1911a) as a digitate frond with digitate fertile tips containing trilete, strongly striated spores, but with no information available concerning the structure of the fertile tips. The material was collected from the Lower Cretaceous Patuxent Formation of the Potomac Group, by Fontaine, who had described this fern under the genus *Baieropsis,* in the Ginkqoales (Fontaine, 1889). He included material in two species of *Baieropsis,* since he misinterpreted the sporangial-bearing structures as fungoid growths on the leaves of a ginkgophyte. Berry (1911a) reassigned the material to the family Schizaeaceae in the Pteridophyta, after illustrating that the fertile tips contained fern spores which were similar to spores of *Anemia* and *Lygodium.*

In 1944, O. Selling studied the spores of the Schizaeaceae and excluded *Schizaeaopsis* from any relationship with *Schizaea* because the fossil had trilete spores and those of *Schizaea* were monolete. Selling suggested that the overall fossil morphology indicated that forms similar to *Schizaea* existed in the early Cretaceous but that lack of details about the fertile parts did not allow unambiguous assignment to the Schizaeaceae.

Recently J. van Konijnenburg-van Cittert (1991) accepted the placement of *Schizaeaopsis* within the Schizaeaceae noting that the macromorphology was like that of *Schizaea/Actinostachys* but the spore morphology was like *Mohria/Anemia.*

Reexamination of the fossil fern has elucidated several additional details that influence its placement within the family and suggest possible relationships with modern genera.

MATERIALS AND METHODS

Specimens of the fossil material are from the Paleobotany Collections at the U.S. National Museum of Natural History, Smithsonian Institution, numbers USNM 3209 and USNM 42459. They are from localities near Fredericksburg collected by W. Fontaine. These beds are dated as Upper Aptian in the Lower Cretaceous (Hickey & Doyle, 1977). The specimens were prepared by degagement (removing the rock matrix with fine pointed needles and hairs). Spores were removed from the specimens and mounted on SEM stubs, coated with gold to 40 nm thickness and viewed with a Hitachi S530 scanning electron microscope at a working distance of 15 mm. Spores from the modern species were removed from herbarium specimens and prepared for SEM in the same way. Spore samples for TEM were embedded in LR White epoxy resin following the recommended procedure, sectioned on a diamond knife and viewed with a JEOL 100 C transmission electron microscope. Material (listed below) used in this study was obtained from the pteridophyte collections in the Botany Department, U. S. National Museum of Natural History, Smithsonian Institution.

Actinostachys pennula (Sw.) Hook. *Liesner and Brewer* 15869
Actinostachys subtrijuga Mart. *Amaral, Cornelio, Guedes & Lima* 523
Actinostachys laevigata Mett. *Noumea* 552
Actinostachys melanesica Selling *Smith* 9592

Schizaea pectinata (L.) Sw. *Werdermann & Oberdieck* 416
Schizaea elegans (Vahl) Sw. *Tryon & Tryon* 5292
Schizaea pusilla Pursh. *Fernald & White* 19513
Schizaea dichotoma (L.) Sm. *Hoogland & Craven* 10690

Description

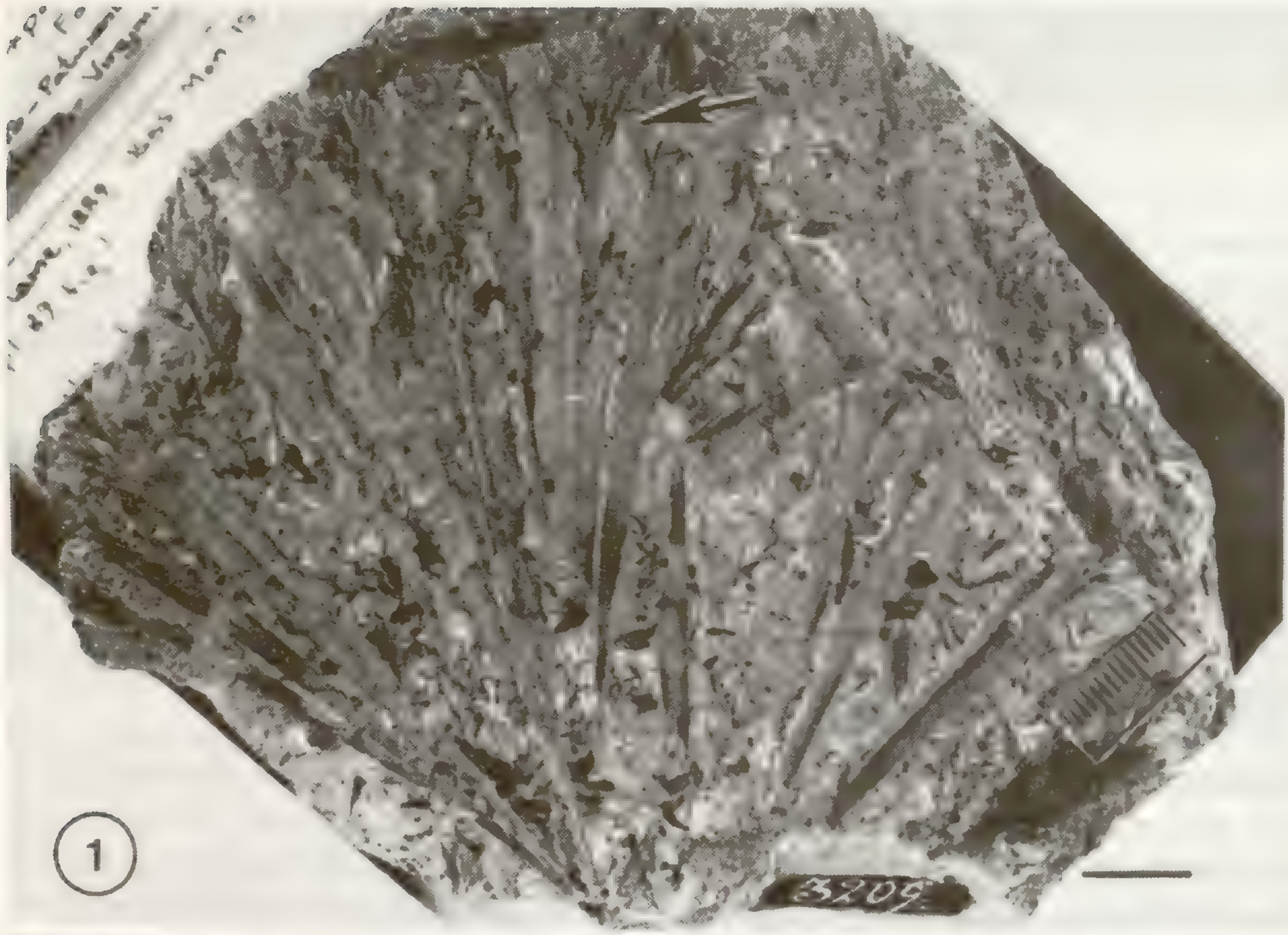
Schizaeaopsis Berry
Annals of Botany, vol. 25, 1911, p. 194
Systematic Paleontology, Lower Cretaceous, Pteridophyta, Maryland Geological Survey, Baltimore, 1911, p. 214.

Schizaeaopsis macrophylla comb. nov.
Baieropsis macrophylla Fontaine 1889, Mon. U. S. Geol. Surv. vol. 15, 1889, p. 212, pl. 90, fig. 6
Baieropsis expansa Fontaine 1889, Mon. U. S. Geol. Surv., vol. 15, 1889, pl. 89, fig. 1
Schizaeopsis expansa Berry 1911, Annals of Botany, vol. 25 p. 194, Textfig. 1, pl. i, figs. 1–6
Schizaeopsis americana Berry 1911, Syst. Paleontol. Lower Cretaceous, Pteriodphyta, Maryland Geol. Surv. Baltimore, p. 214, fig. 2, pl. 22, figs. 1–9.

Emended Diagnosis: Fronds repeatedly dichotomous from the base, veins also dichotomous. Fertile portions oval, mainly apical or occasionally at vein endings along the pinnule margins, fertile tips pinnately divided, sporangia borne along these divisions on both sides of midvein, tightly packed together, annulus apical. Spores tetrahedral, trilete, laesura raised, 50–80 μm in diameter, striated, parallel muri 2 μm wide, occasionally dividing, grooves 0.5 μm wide; spherules of perispore material scattered on surface, fine granular layer of perispore material over surface striations.

Description: The fossil material consists of flattened fronds 6–8 cm long and 4–6 cm wide at the apex that dichotomize several times (Fig. 1, 2 & 20). All bear the fertile tips at vein endings, mainly at the ends of the dichotomous divisions, but these tips are not always all at the same level on the frond (Fig. 3 & 20a). The veins run parallel through the lamina and also occasionally divide (Fig. 4). Veins, or a division from a vein if the fertile tip is below the apex, enter the fertile tips (Fig. 5 & 20b). Unlike the sterile frond segments the fertile tips (3–4 mm long, 1 mm wide) have a central rachis with pinnate lobes (Fig. 6). The pinnate lobes are somewhat folded, due to either maturity or preservation. Removal of the upper layer of folded pinnate lobes reveals that the sporangia are closely packed on the surface of the fertile tips, (Fig. 7), are 0.3–0.5 mm in diam. and have an apical annulus. Spores have been isolated from the sporangia, as noted by Berry (1911b). They are tetrahedral, trilete, have a diameter of 50–80 μm and are ornamented with muri 1–2 μm wide and grooves 0.5 μm wide in the exospore (Fig. 8 & 9). The laesura is slightly raised and often one of the arms is shortened (Fig. 10). The spores have small granular particles (spherules) scattered over the surface; this is the perispore material (Fig. 8–10). The perispore also forms a fine granular layer covering the exospore (Fig. 11).

Type: USNM Paleobotany Collection 3209
Locality: Fredericksburg, VA Patuxent Formation, Potomac Group
Age: Upper Aptian, Lower Cretaceous



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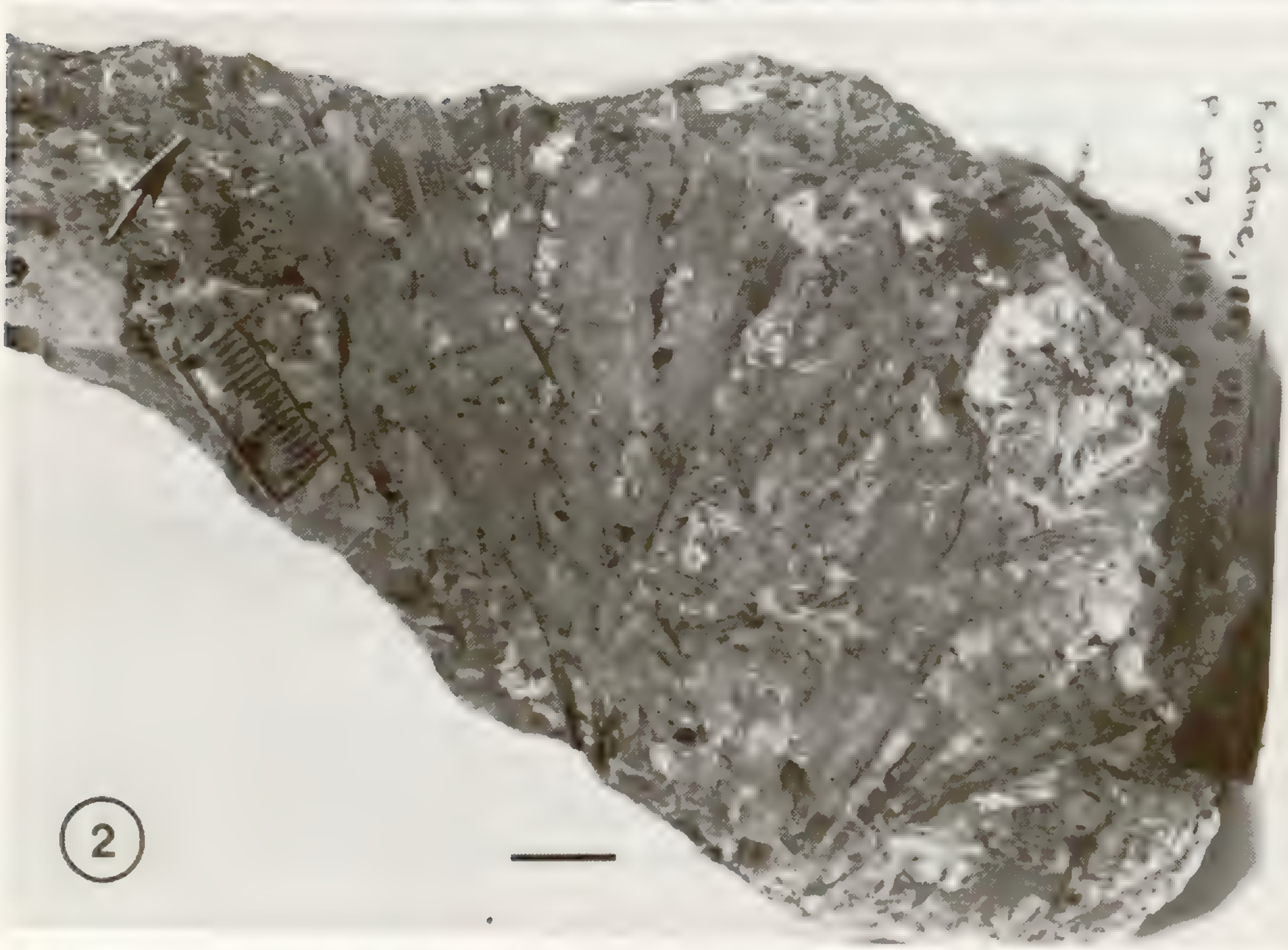


2

Fig. 1. *Schizaeaopsis macrophylla* USNM 3209. natural size. Dichotomous frond shown with fertile portions at top, arrow indicates one fertile region. Scale bar = 1 cm.
Fig. 2. *Schizaeaopsis macrophylla* USNM 42459. Natural size. Dichotomous frond with one fertile tip shown at left of specimen at arrow. Scale bar = 1 cm.

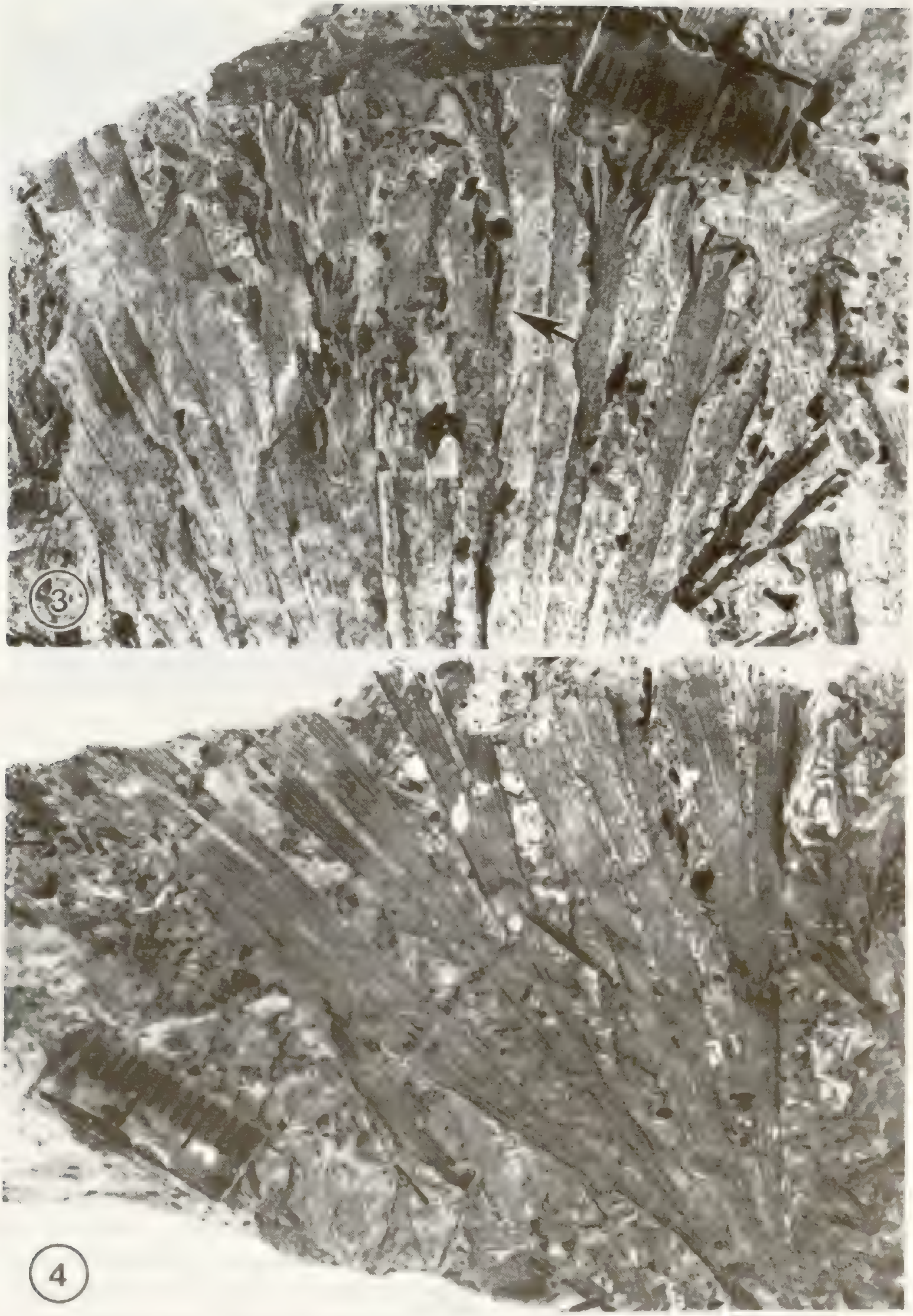
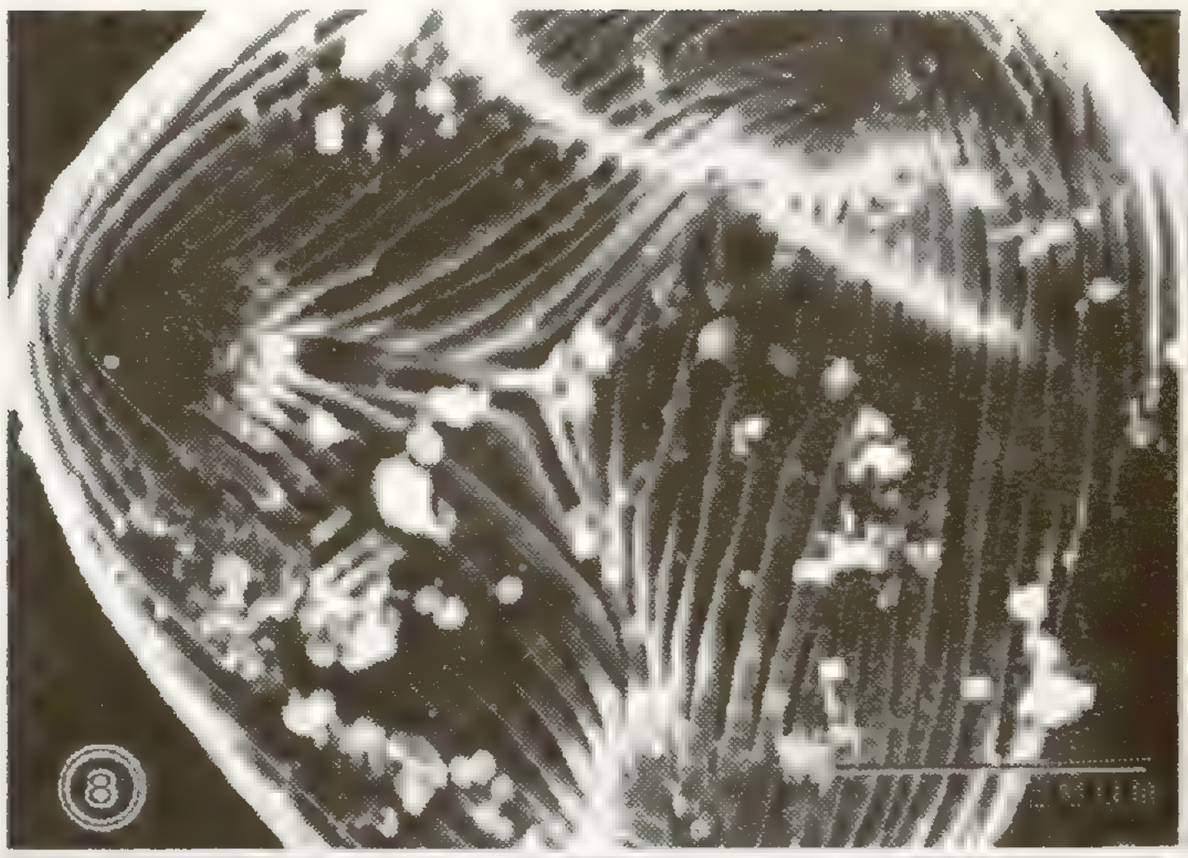


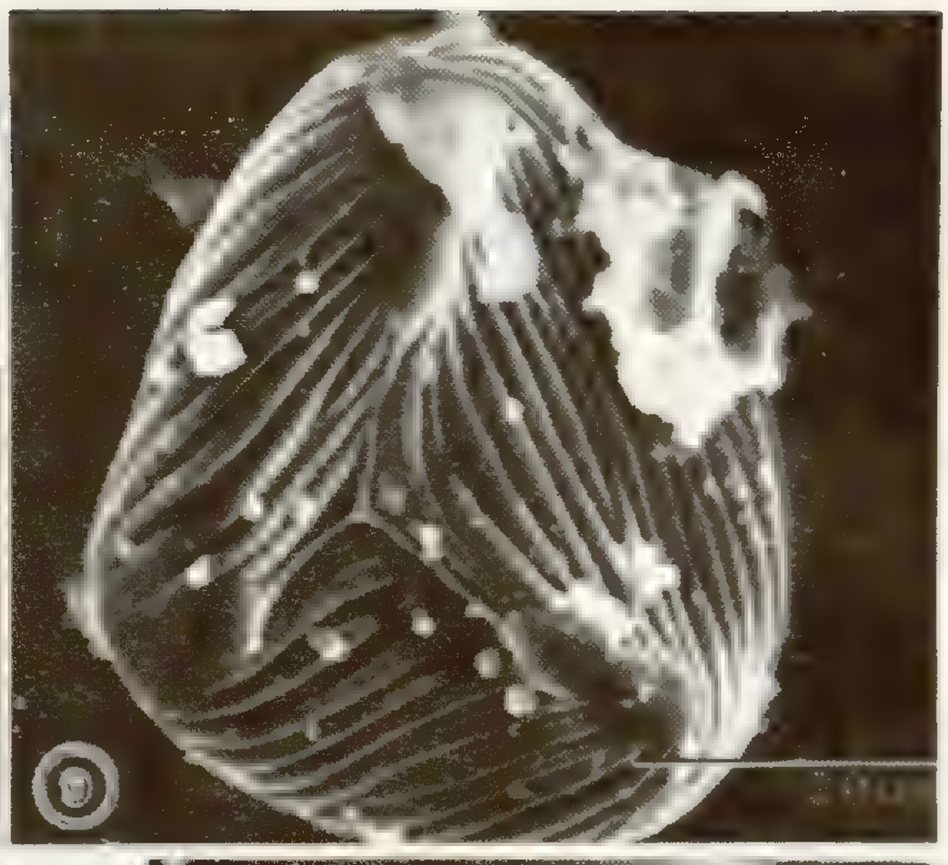
Fig. 3. USNM 3209 at twice natural size showing fertile tips terminating frond divisions. Although most arise at nearly the same level, some (arrow) are produced at different levels near the tip. Divisions on scale are 1 mm.
Fig. 4. USNM 42459 at twice natural size to show dichotomies and venation of frond. Divisions on scale are 1 mm.

Fig. 5. USNM 3209. One fertile tip of digitate frond portion with vein extending up to fertile division (arrow). Top portion is broken, but most of the fertile divisions attach at nearly the same point. Scale bar = 1 mm.
Fig. 6. One fertile division (USNM 42459) showing pinnate subdivisions (arrow at one). Right side shows pinnate divisions to tip, but left side divisions are folded below into the rock. Scale bar = 0.5 mm.
Fig. 7. USNM 42459 showing sporangia with apical annuli (arrows) over surface of pinnate divisions. Scale bar = 0.25 mm.

Discussion

The genus *Schizaeopsis* was established by Berry (1911a) based on several specimens collected by Fontaine. Fontaine had assigned them to the genus *Baieropsis* (1889) on the basis of the leaf morphology. Fontaine described the sporangia-bearing structures as a fungoid growth on the leaves in the species *B. macrophylla* (USNM 3209 is the illustrated specimen), but made no mention of them on the specimen assigned to *B. expansa* nor is any fertile material of that species illustrated by Fontaine. Berry (1911a) combined *B. macrophylla* with one specimen of *B. expansa* that did bear sporangia (USNM 42459), identified the terminal tips as bearing sporangia and on the basis of the spore characteristics assigned them to *Schizaeopsis* under the name *S. expansa*. Later that year Berry (1911b) noted that "since *Baieropsis expansa* was the type of Fontaine's genus *Baieropsis* it cannot be made the type of the new genus *Schizaeaopsis*", although Fontaine never designated a type for his genus (see Index Nominum Genericorum). Therefore, Berry assigned a new specific epithet, *S. americana,* to the material he had transferred from *Baieropsis*. Since he transferred only one specimen from *B. expansa* and that specimen is distinct from *B. expansa* as described by Fontaine, then the species name should not be *S. expansa*. The specimen illustrated by Berry in his publication is the specimen that Fontaine had identified as *Baieropsis macrophylla*. Berry also transferred all the material Fontaine had originally assigned to *B. macrophylla* to the new genus *Schizaeaopsis*. Thus





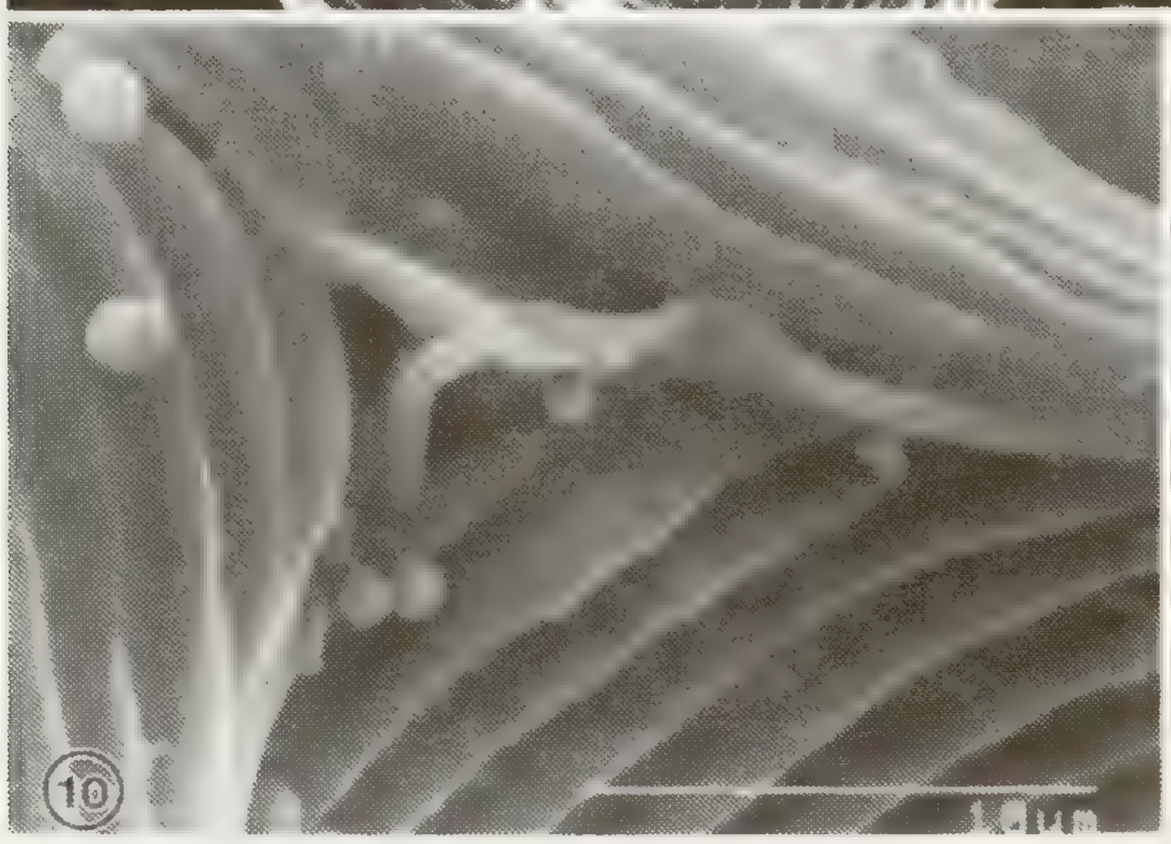


Fig. 8. SEM of spore from USNM 3209. Proximal view of trilete laesura and muri and grooves. Perispore spherules on surface.
Fig. 9. SEM of spore from USNM 42459.
Fig. 10. SEM of spore from USNM 3209 showing shortened arm of the trilete mark, and perispore spherules.
Fig. 11. SEM of muri showing very thin granular layer of perispore on surface of exospore. Scale bar = 0.5 μm.

the older specific epithet available for the material now in *Schizaeaopsis* is actually *S. macrophylla* and the name *S. americana* becomes superfluous. The proper designation is made here, and the type specimen is designated by USNM number (USNM 3209) to clarify the situation.

Comparison with modern ferns clearly indicates relationships to *Schizaea* and *Actinostachys.* The vegetative morphology of *Schizaea* is a digitate frond with a fertile tip on the digitate divisions. In most species there is only one fertile portion on a frond division. Each of the fertile tips is once pinnately divided. In mature *Actinostachys* there is a group of fertile digits on a single frond division attached nearly at a common point (Bierhorst, 1969). Individual digits of *Actinostachys* bear more sporangia than *Schizaea,* in four rows instead of two, and the sporangia are protected by an indusial flap. In *Schizaea* the folding of the pinnate unit protects the young sporangia (Bierhorst, 1969). Bierhorst also suggested that the multipinnate condition of the fertile unit is primitive.

The fossil fern has characteristics of each of the two modern genera. The fertile tips are once pinnately divided and folded over as in *Schizaea,* but there are many fertile digits at the tip of the laminar unit; some are attached nearly at a common point as in *Actinostachys* whereas others may be attached lower down on the lamina. While there is

Fig. 12. SEM of spore of *Actinostachys pennula*. Leisner & Brewer 15869. Thin granulate perispore over striate endospore. Scale bar = 10 μm.
Fig. 13. SEM of spore of *Actinostachys subtrijuga*. Amaral, Cornelio, Guedes & Lima 523. Thin perispore with few spherules over striate exospore. Monolete laesura at top. Scale bar = 10 μm.
Fig. 14. SEM of spore of *Schizaea pusilla*. Fernald & White 19513. Granulate perispore with many spherules. Scale bar = 10 μm.
Fig. 15. SEM of spore of *Schizaea elegans*. Tryon & Tryon 5292. Thicker granulate perispore with spherules obscuring exospore. Scale bar = 10 μm.

little question that the frond morphology is similar to the modern genera, the spore morphology is different. Clearly the trilete, tetrahedral, striate spores of *Schizaeaopsis* are closer in morphology to those of *Anemia* subgenus *Anemiorhiza* than to the monolete, reniform spores of *Schizaea* (see Tryon & Lugardon, 1991; van Konijnenburg – van Cittert, 1991). The resemblance of *Actinostachys* spores to those of *Anemia* was noted by Tryon and Lugardon (1991) even though the first is monolete and the latter is consistently trilete. The only trilete spores reported in *Schizaea* are the rare ones in *S. dichotoma* seen by Selling (1944), who excluded *Schizaeaopsis* from any relationship with the modern genus on the basis of the trilete spores, suggesting the fossil form only indicated that *Schizaea* – like morphology had appeared at that time. However, van Konijnenburg-van Cittert (1991) accepted its relationship and uses the evidence from the spores that the earliest spores of this lineage were trilete and striate and monolete spores that are scabrate, granulate and alveolate evolved later.

In an attempt to compare the fossil spores to the modern ones several species of *Schizaea* and *Actinostachys* were examined by scanning and transmission electron

Fig. 16. SEM of *Actinostachys laevigata* Noumea 552, spore showing granulate perispore on mature spore.
Fig. 17. SEM of *Actinostachys laevigata* Noumea 552, spore in area where perispore has been removed showing striated exospore below.
Fig. 18. TEM of *Actinostachys pennula* Lorimer & Brewer 15869, showing thick striate exospore and more darkly stained thin perispore (arrow). Scale bar = 0.5 µm.
Fig. 19. TEM of young *Actinostachys laevigata* Noumea 552, spore showing striate exospore and areas of thickening perispore around and over the grooves (arrows). Scale bar = 0.5 µm.

microscopy. Most of the spores conform to the general observation (van Komjnenburg-van Cittert, 1991) that *Actinostachys* spores have an exospore of parallel ridges and furrows (Fig. 12 & 13) with a thin perispore that is easily removed and *Schizaea* spores have a smooth or more or less granulate exospore with a granulate perispore (Fig. 14 & 15). One species of *Actinostachys*, however, showed a developmental sequence that was particularly informative. *A. laevigata* has spores in the older sporangia that are granular (Fig. 16), but when the perispore is broken away (Fig. 17) the striae are visible below in the exospore. Most *Actinostachys* spores in section show a striated exospore with a thin layer of granulate perispore (Fig. 18). When the spores from the younger sporangia of *A. laevigata* are sectioned the exospore is striate with mum and grooves, as occurs in most species of *Actinostachys*. Gradually deposits of perispore material are laid down (illustrations in Tryon & Lugardon, 1991; van Komijnenburg van Cittert, 1991) and these fill in the grooves and cover the striae (Fig. 19) until the exospore ornamentation is completely covered by the perispore. This developmental sequence suggests that the transition from

Fig. 20. Line drawing of specimen USNM 3209 showing different levels of fertile tips and dichotomies (a) and an enlargement (b) to show veins and pinnate divisions of fertile tips (see Fig. 5). Scale bars a = 1.0 cm, b = 1.0 mm.

the striate spores to granular spores and then to other modifications is a result of loss of exospore ornamentation and gain of perispore granular material. The exospore is smooth or granulate in extant *Schizaea* (Tryon & Lugardon, 1991), and perispore is very sparse in the fossil spores and in the striate *Actinostachys* spores (van Konijnenburg-van Cittert, 1991).

The fossil fern *Schizaeaopsis* thus combines characteristic spores of *Anemia/Mohria* with frond morphology of *Schizaea/Actinostachys.* During the same time period (Aptian) the first *Actinostachys* – like dispersed spores occur (van Konijnenburg – van Cittert, 1991). The Lower Cretaceous Potomac Group also has fossils belonging to the *Anemia/Mohria* complex and these appear from slightly older beds (Skog, 1987, 1991). They appear to be characterized by more primitive features of *Anemia* according to the character analysis of Mickel (1962) for the genus. The Lower Cretaceous therefore is likely a time of divergence of the common ancestor of *Actinostachys/Schizaea* from *Anemia.* The modern taxa *Actinostachys* and *Schizaea* probably became distinct during the Tertiary. Spores like *Actinostachys* continue from Upper Cretaceous through Paleocene and Eocene, but spores like *Schizaea* first appear in the Miocene (Selling, 1944).

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Antheridiogen Response In *Phanerophlebia* and Related Fern Genera

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Antheridiogens are naturally-occurring plant products that act pheromonally to affect sexual expression in the gametophytes of some pteridophyte species. The production of an antheridiogen was first reported by Döpp (1950), who observed that developing gametophytes of *Pteridium aquilinum* (L.) Kuhn produced a substance that caused other, newly germinated gametophytes of this species to produce antheridia precociously and abundantly, with a subsequent delay in the formation of a meristematic notch and archegonial production. Since that time, the action, expression, and biochemical structure of this type of compound have been studied in some detail (for reviews see Voeller, 1964; Näf et al., 1975).

Subsequent research has also demonstrated that three biochemically distinct general classes of antheridiogens are produced by taxonomically diverse species, best identified trivially by the capital letters A, B, and C, to denote biologically interreactive groupings (Schedlbauer and Klekowski, 1972; Haufler and Gastony, 1978). Antheridiogen A, first isolated from *P. aquilinum* (Döpp, 1950), affects gametangial development in gametophytes of some members of such diverse families as Blechnaceae, Cyatheaceae, Davalliaceae, Dennstaedtiaceae, Dicksoniaceae, Dryopteridaceae, Polypodiaceae, Pteridaceae, and Thelypteridaceae (all *sensu* Tryon and Tryon, 1982) (Näf et al., 1975; Voeller, 1964). Antheridiogen B, first demonstrated in *Anemia phyllitidis* (L.) Sw. (Näf, 1959), affects several species of Schizaeaceae. Antheridiogen C is specific to *Ceratopteris* (Pteridaceae; Schedlbauer and Klekowski 1972). Fern species tested for antheridiogen response were reviewed by Näf et al. (1975), with subsequent additions by Schedlbauer and Klekowski (1972), Haufler and Gastony (1978), and Haufler and Ranker (1985).

Antheridiogens have been implicated as functioning in nature to promote outcrossing in pteridophytes by mediating obligate cross-fertilization through ontogenetic control of gametangial formation in natural gametophyte populations (see Schneller et al., 1990, for review). Previous studies have emphasized two different approaches to the elucidation of antheridiogen function in ferns. As noted above, several studies have documented the susceptibility of gametophytes of various species to antheridiogens of "classical" producers, as well as the production of native antheridiogens by many of these same species under laboratory culture conditions. These studies have involved exogenous hormonal application by germination of spores on antheridiogen-enriched nutrient agar. Other studies have characterized populations of gametophytes in the field to demonstrate that naturally occurring gametophytes of some temperate and tropical pteridophytes do exist as unisexual plants, as would be predicted if an antheridiogen system were operative in nature (Tryon and Vitale, 1977; Schneller, 1979; Farrar and Gooch, 1975). There have not been any studies to confirm directly the action of an antheridiogen system in nature and problems of experimental design and adequate controls may be insurmountable in this regard. However, recent studies have suggested the existence of an antheridiogen system

in nature by demonstrating that naturally occurring populations in some species of *Bommeria* (Pteridaceae; Haufler and Soltis, 1984) and *Hemionitis* (Pteridaceae; Schneller et al., 1990) that are known to produce a native antheridiogen under laboratory conditions are also highly outcrossing. Other studies have also demonstrated correlations between increased genetic load and antheridiogen production (Schneller et al., 1990).

In spite of the apparent importance of antheridiogens in mediating breeding systems, there have been relatively few studies to determine the extent of antheridiogen production and susceptibility among fern species. Soltis and Soltis (1987) recently noted that less than 1% of all fern species have been tested for antheridiogen response. The present study was undertaken during general biosystematic research on the closely related genera *Cyrtomium, Phanerophlebia,* and *Polystichum* (Dryopteridaceae) to document the production of and/or susceptibility to antheridiogens of representative taxa under culture conditions. A clarification of the breeding system operating in these plants might contribute to an understanding of levels of genetic variation that exist within and between populations and, indirectly, to an understanding of systematic relationships and evolution within the group.

Polystichum is a nearly cosmopolitan genus of ca. 160 species (Tryon and Tryon, 1982). Several segregates of this large and variable genus have been described, the largest of which are the Asiatic *Cyrtomium* and the neotropical *Phanerophlebia.* In spite of the fact that several species of *Polystichum* are relatively widespread in various parts of the world, and that other species of this genus, as well as of *Cyrtomium,* are commonly cultivated for horticultural purposes, relatively few members of this complex have been examined for antheridiogen production. *Polystichum acrostichoides* (Michaux) Schott (Näf, 1956; Voeller, 1964) and *P. tsus-simense* (Hook.) J. Smith (Näf, 1969) previously have been shown susceptible to various concentrations of antheridiogen A. *Cyrtomium falcatum* (Thunb. ex L. f.) C. Presl was found to be unaffected by antheridiogen A, as was *P. tsus-simense* at lower concentrations of the hormone (Voeller, 1964). *Cyrtomium falcatum* is notable among the previously tested taxa in existing in nature primarily as an apomict, a fact not addressed by previous studies.

Material and Methods

Species surveyed in this study are listed in Table 1. Spores were collected from greenhouse-grown plants by rinsing portions of fertile fronds with distilled water and pressing between sheets of writing paper. Spore samples were sterilized and grown axenically (Haufler and Gastony, 1978). After ca. ten weeks of growth, mature gametophytes were scraped from each culture with a spatula. Agar samples were diluted with fresh agar (by weight) to yield 0.5 and 0.25 dilutions, autoclaved, repoured, and resown with appropriate sterile spores, as above. Germination and development of these latter spore samples were observed under the dissecting microscope at one week intervals for ten weeks, to record gametophyte development and ontogeny of gametangia for each species.

Pteridium aquilinum supplied antheridiogen A, with *Onoclea sensibilis* L. used as a control for the activity of this antheridiogen. Spores of each species samples (Table 1) were sown on agar containing antheridiogen A at both concentrations, to test for susceptibility to this general class of antheridiogens. Each sample species was also tested for production of a native antheridiogen, both by observing its growth on its own agar source and by observing growth of *Onoclea* gametophytes on agar from each species.

Table 1. Samples used in this study. Pressed vouchers are accessioned at IND and include more complete locality data. Samples are sexually reproducing taxa, except where noted.

Species	Origin of Sample
PHANEROPHLEBIA	
P. auriculata Underw.	ARIZONA: Cochise County; *Yatskievych 83-161*
P. juglandifolia (Humb. & Bonpl. ex Willd.) J. Smith (diploid)	MEXICO: Edo. Chiapas; *Yatskievych et al. 85-182*
P. juglandifolia (tetraploid)	COSTA RICA: Prov. San José *Yatskievych & McCrary 86-13*
P. macrosora (Baker) Underw.	COSTA RICA: Prov. Heredia; *Yatskievych & McCrary 86-30*
P. nobilis (Schlecht. & Cham.) C. Presl	MEXICO: Edo. México; *Yatskievych et al. 85-211*
P. pumila (Mart & Gal.) Fée	MEXICO: Edo Oaxaca; *Yatskievych et al. 85-139*
P. remotispora (Fourn.) Underw.	MEXICO: Edo. Hidalgo; *Yatskievych et al. 83-353*
P. umbonata Underw.	MEXICO: Edo. Nuevo León; *Yatskievych & Wollenweber 83-87*
CYRTOMIUM	
C. falcatum (Thunb. ex L. f.) C. Presl (apomictic)	SOUTH CAROLINA: Charleston County; *Yatskievych & McCrary 83-184*
C. fortunei J. Smith (apomictic)	SOUTH CAROLINA: Charleston County; *Yatskievych & McCrary 83-185*
C. macrophyllum (Makino) Tagawa (apomictic)	JAPAN: Honshu, Shiga Pref., *Mitsuta in 1984*
POLYSTICHUM	
P. acrostichoides (Michx.) Schott	INDIANA: Monroe County; *Yatskievych & McCrary 83-258*
P. imbricans (D. C. Eaton) D. H. Wagner ssp. *curtum* Ewan) D. H. Wagner	CALIFORNIA: Riverside County; *Yatskievych & McCrary 84-119*
P. lonchitis (L.) Roth	ARIZONA: Coconino County; *Yatskievych & WIndham 85-302*
P. munitum (Kaulf.) C. Presl	CALIFORNIA: Marin County; *Yatskievych & McCrary 84-131*
MISCELLANEOUS TAXA	
Pteridium aquilinum (L.) Kuhn var. *latiusculum* (Desv.) Heller	INDIANA: Monroe County; *Yatskievych & McCrary 83-259*
Onoclea sensibilis L.	INDIANA: Brown County; *Yatskievych & Johnson 84-178*

Results and Discussion

Spore germination and gametophyte development and morphology in the three genera under study are the typical types found in the family Dryopteridaceae (Nayar and Kaur, 1971). Gametophyte development in selected species of *Cyrtomium* and *Polystichum* was described by Chandra and Nayar (1970) and is in general agreement with observations made during this study. For all species in the three genera, mature gametophytes are cordate and copiously glandular with unicellular trichomes. The normal sequence of gametangial development for isolated gametophytes in the sexual taxa involves produc-

tion of archegonia soon after development of a meristematic notch (7–8 weeks after spore germination), at which point prothallia consist of ca. 100–150 cells. Antheridial production follows after 1–2 additional weeks of growth. For the three apomictic species (Table 1) no archegonial development was observed in any of our cultures and antheridial formation was both tardy and irregular.

Susceptibility to antheridiogen A and production of a native antheridiogen for all samples are reported in Table 2. All controls performed as expected. Reduced growth of *Onoclea* gametophytes on 50% *Pteridium* agar, as had been noted by Haufler and Gastony (1978), was not observed during this study, probably due to slightly differing concentrations of antheridiogen and other solutes in the agar stocks. For the sexual taxa analyzed, all samples responding to antheridiogen A also produced a native antheridiogen. That these native compounds correspond to the antheridiogen A type is demonstrated by the fact that gametophytes of *Onoclea* responded to them in all cases. For *Phanerophlebia nobilis* the response of sample gametophytes to both native antheridiogen and antheridiogen A from *Pteridium* was slight and was indicated only by the slightly precocious production of greater numbers of antheridia in comparisons of sample and control plates. Previous studies have documented infra- and interpopulational variation in levels of antheridiogen sensitivity for various species (Schneller et al., 1990). Thus, further tests to determine whether antheridiogen susceptibility varies between populations of this and other *Phanerophlebia* species would be instructive.

The present study confirms previous findings (Voeller, 1964) that *Polystichum acrostichoides* responds to antheridiogen A, whereas the apomictic *Cyrtomium falcatum* does not. Gametophytes of *C. fortunei* and *C. macrophyllum,* however, were found to produce a native antheridiogen (Table 2), as shown by the response of *Onoclea* gametophytes when grown on agar from these species. However, neither of these taxa showed any response to antheridiogen A, nor did either respond to its own native antheridiogen (gametophytes on treatment agar showed growth rates and unresponsive, cordate morphologies identical to those grown simultaneously on unenriched agar). This unusual observation presumably relates to the apomictic life cycles of these taxa and correlates with the observed irregular production of antheridia by prothallia of these plants. It is not, however, the only type of antheridiogen response possible for apomictic taxa. For the apogamous *Bommeria pedata* (Sw.) Fourn., Haufler and Gastony (1978) showed normal levels of archegonial and antheridial production and strong response to antheridiogen A, in spite of the redundancy of such a pherormonal response in an apomictic plant. If the inference of these authors is correct, that *B. pedata* is a recently evolved apomict because it retains its antheridiogen susceptibility, then the data for the *Cyrtomium* species suggest that they are of less recent origin, because they *have* lost their antheridiogen susceptibility, but continue to produce a native compound.

As outlined by Willson (1981), production of an antheridiogen also might convey a potential selective advantage for such taxa through a kind of allelopathic mechanism. Although the pheremone would have no effect on development of neighboring gametophytes of the same species (which are not susceptible), it would inhibit growth and production of archegonia in prothallia of adjacently growing gametophytes of other fern species that do respond to an antheridiogen. These gametophytes presumably would compete with the *Cyrtomium* gametophytes for space and nutrients. Such inference, however, remains purely speculative at this point.

Table 2. A summary of tests for response of sample taxa to two concentrations of antheridiogens (see text for details).

species	*response to Antheridiogen A* 50%	25%	*production of native antheridiogen* 50%	25%
PHANEROPHLEBIA				
P. auriculata	-	-	-	-
P. juglandifolia (2x)	-	-	-	-
P. juglandifolia (4x)	-	-	-	-
P. macrosora	-	-	-	-
P. nobilis	+	+	+	+
P. pumila	-	-	-	-
P. remotispora	+	+	+	+
P. umbonata	+	+	+	+
CYRTOMIUM				
C. falcatum	-	-	-	-
C. fortunei	-	-	+	+
C. macrophyllum	-	-	+	+
POLYSTICHUM				
P. acrostichoides	+	+	+	+
P. imbricans ssp. *curtum*	-	-	-	-
P. lonchitis	-	-	-	-
P. munitum	-	-	-	-

Antheridiogen response appears to be a poor character for defining natural lineages within the polystichoid ferns, since reaction to these compounds is sporadic among species in various groups in this assemblage. Within *Planerophlebia,* however, the species exhibiting susceptibility to antheridiogen A are quite closely related. Recent studies comparing the chloroplast genomes of *Phanerophlebia* species though analysis of restriction site mutations have provided evidence that *P. nobilis* and *P. remotispora* are probably best treated as conspecific varieties of *P. nobilis* (Yatskievych et al., 1988; Yatskievych, 1990). These studies also have shown that *P. nobilis* is a potential diploid progenitor of allotetraploid *P. auriculata. Phanerophlebia umbonata* is probably the other progenitor of *P. auriculata,* based on morphological and biogeographic considerations (Yatskievych, 1990). Thus, the lack of antheridiogen response in tetraploid *P. auriculata* is puzzling, in light of the response of both presumed progenitor taxa.

In contrast to the recent work of Haufler and Soltis (1984) on *Bommeria* gametophytes, the present laboratory findings do not correlate with field-based research on breeding systems of *Phanerophlebia* species. Allelic frequencies for the five diploid species compiled during a recent study of allozyme polymorphisms (Yatskievych, 1990) were subjected to analysis of inbreeding rates using techniques and a computer program developed by Kent Holsinger (1987). The maximum likelihood and bootstrap estimates and confidence intervals are summarized in Table 3. There is no significant difference in inbreeding rates between the antheridiogen producing taxa and those that neither produced nor responded to an antheridiogen. All species are strongly outcrossing. Therefore, at least in the genus *Phanerophlebia,* antheridiogens are not the primary factor in determination of

Table 3. Intragametophytic selfing rates for five diploid species of *Phanerophlebia* calculated from isozyme data (Yatskievych, 1990), using maximum likelihood and bootstrap estimates (Holsinger, 1987). Values in parentheses are 95% confidence intervals.

Species	Antheridiogen Production	Maximum Likelihood Estimates	Bootstrap Estimates
P. juglandifolia	-	0.0919 (0.0000-0.2549)	0.1127 (0.0000-0.2476)
P. macrosora	-	0.0108 (0.0000-0.3365)	0.0000 (0.0000-0.2481)
P. nobilis	+	0.0000 (0.0000-0.1690)	0.0000 (0.0000-0.1559)
P. remotispora	+	0.0000 (0.0000-0.1491)	0.0000 (0.0000-0.1280)
P. umbonata	+	0.0000 (0.0000-0.2411)	0.0000 (0.0000-0.1870)

the breeding system. Whether antheridiogens are important in intergametophytic competition for space and resources (see discussion above) remains to be established by future research.

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Application of the Name *Goniophlebium* and a New Subgeneric Name in *Polypodium*

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In publishing the new generic name *Goniophlebium,* K. Presl (1836, pp. 185–186) provided a description based on five New World species, which he transferred to his genus. He also included the apparent synonym "Polypodium spuriorum sectio 1, Goniophlebium. *Blume,*" which was based on three Old World species. (On examining Blume's work (1830, p. 132), one finds this "section" to be a rankless name, but one valid under Art. 35.2 and so available for transfer.) He also transferred each of Blume's species with a query.

Presl's concept clearly applied to American material, for he saw no Asiatic material and he included Blume's name and species only "ex auctoritate clar. Blume . . ." It is very likely that Presl intended to establish a name independent of Blume's, as the preponderance of evidence indicates: American species described and transferred without doubt, Asiatic species not described, transferred with doubt, and only on the authority of Blume. J. Smith (1841, p. 56; 1875, p. 92) recognized the difference between New World and the Old World species. He mentioned their articulate pinnae and proposed that they be placed in *Goniophlebium* sect. *Schellolepis* or in *Schellolepis.* Pichi Sermolli (1973, pp. 465–468) agreed with that disposition, chose one of Presl's American species as lectotype, and cited the genus as *Goniophlebium* K. Presl.

Had Presl excluded Blume's name (by not citing it) or Blume's species (by piacing them in another genus), there would be no problem in considering Presl's name to be independent of Blume's. However, according to the present Code (Arts. 48.1, 63.2), by including Blume's name and his species – even with doubt – it is mandatory to base Presl's name on Blume's, to choose a lectotype from among Blume's species, and to cite the genus as *Goniophlebium* (Blume) K. Presl, as Rödl-Linder et al. (1990, p. 105) recently did. Only an act of conservation can override this disposition.

In my opinion, the American species are different from the Asiatic ones, most notably in lacking articulate pinnae and in having sori in several series, as well as in a single series. These species seem best retained in a subgenus of *Polypodium* (Lellinger, 1981, p. 93). Hennipman, Veldhoen and Kramer (1990, pp. 205–206, 224–225) held the same viewpoint; they pointed out several hybrids that occur between the subgenera of American *Polypodium.* In addition, they illustrated dissected lamina scales and paraphyses of *Polypodium (Goniophlebium) verrucosum* (Hook.) J. Smith, neither of which I have observed in New World species, whose laminae are rarely pilosulous and perhaps never paleaceous or paraphysate.

Because the names *Goniophlebium* (Blume) K. Presl and *Polypodium* subg. *Goniophlebium* (Blume) C. Chr. must be applied to Old World material that is not clearly allied to material from the New World, the New World subgenus traditionally called *Polypodium* subg. *Goniophlebium* requires a new name:

Polypodium subg. **Polygoniophlebium** Lellinger, subg. nov.

Rhizoma late repens, frondibus distantibus; laminae pinnatae pinnatisectae vel pinnati-

fidae, pinnis non articulatis, pinna terminalis conformis vel apice laminae pinnatifido; areolae fertilia in seriebus (1)2–5(8) dispositae; venae primariae pinnatae parallelae; venae secundariae angularae, venulis inclusis simplicibus excurrentibusque.

TYPE: *Polypodium fraxinifolium* Jacq. *(Polypodium articulatum* Desv. and *P. rhizocaulon* Willd., two of Presl's original species, are synonyms of *P. fraxinifolium.)*

The species of sect. *Polygoniophlebium* are by no means uniform. The type species, along with species such as *P. adnatum* Kunze and *P. giganteum* Desv., have long rhizomes with appressed scales, large, pinnate laminae, and sori typically in 3–6 series. Similarly large plants with shorter rhizomes, pinnatifid or pinnatisect laminae (at least toward the apex), and sori typically in 1–4(5) series include *P. attenuatum* Humb. & Bonpl. ex Willd., P. *kuhnii* Fourn., and *P. triseriale* Swartz. One small and apparently isolated group of species has short rhizomes and dark-green laminae with one row of slightly elongate sori; it includes *P. plectolepidioides* Rosenst. and *P. rhachypterygium* Liebm. Two groups have smaller, mostly pinnatifid laminae with a single row or rarely two rows of sori on each side of the costa. Those with very long-creeping rhizomes and usually appressed scales include *P. loriceum* L., *P. catharinae* Langsd. & Fisch., *P. maritimum* Hieron., and *P. ptilorhizon* Christ. Those with shorter rhizomes and spreading scales include *P. dissimile* L., *P. plesiosorum* Kunze, and *P. wagneri* Mett. *Polypodium sessilifolium* Desv. is similar, but is fully pinnate.

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SHORTER NOTE

First continental record of *Thelypteris guadalupensis* (Wikström) Proctor in the state of Quintana Roo, Mexico. — As a result of intensive field work in the southern part of the State of Quintana Roo, Mexico, *Thelypteris* (subgenus *Goniopteris) guadalupensis* (Wikström) Proctor was found in the Municipio Othón P. Blanco *(S. Torres* et al. *605,* CIQRO, UAMIZ; *Ceballos 1,* CIQRO), growing on limestone rocks and associated with *Tectaria heracleifolia,* in low subdeciduous forest. The species is very rare and only two mature specimens and a few small plants have been found in the site of recollection. The collections represent the first continental record of the species in America, otherwise widely distributed in Greater and Lesser Antilles; *Thelypteris guadalupensis* is one of the few species of the genus with pinnatifid or merely lobed leaf, and it differs from the endemic *T. hildae* Proctor from Puerto Rico, for the shorter petiole, 20% of the leaf length of shorter, blades decrescent downward with 2–5 distinct pairs of somewhat reduced pinnae and minutely stellate-puberulous tissue in the abaxial side of the leaf. The comments of Alan R. Smith about the identification of the plants are gratefully acknowledged. —RAMON RIBA, UAM-Iztapalapa; Ap. Postal 55-535, México, D.F. 09340, and S. TORRES, CIQRO; Ap. Postal 424, Chetumal, Q. Roo 77000.

REVIEWS

Ferns and orchids of the Mariana Islands by Lynn Raulerson and Agnes F. Rinehart. 1992. 138 pp. Available from the authors, P. O. Box 428, Agana, Guam 96910. Paper, $15.00 postpaid. ISBN 1-878453-09-2.

Guam, Saipan, and Tinian are best known of the 15 Mariana Islands, which form a gentle north-south arc in the western pacific Ocean about one-third of the way between the Philippine Islands and the Hawaiian Islands. These low islands have both volcanic and coral-derived soils. The climate is tropical maritime, with a 3-month dry season at the beginning of the year and 5-month wet season that ends just before the end of the year. The pteridophytes number 90 species, excluding exotics that have not become naturalized. As is common in the floras of micronesian islands, the species are mostly those that disperse and establish themselves well, especially of *Thelypteris,* as well as *Nephrolepis, Polypodium,* and *Pteris.* The Marianas have a surprisingly diverse pteridophyte flora, everything from *Ophioglossum* to *Cyathea* to *Schizaea.* Virtually every species is illustrated, most by handsome habit and detail photographs. In a few cases, when the plants grew on outlying islands or otherwise could not be photographed in situ, a herbarium specimen was photographed. In all cases, the photographs are diagnostic. Keys are provided only to the species of larger genera. Each species' range and habitat are given and it is briefly described. Miscellaneous notes on uses, cultivation, etc. are included for each species. Some dubious or provisional species are mentioned at the conclusion of the fern treatment to give readers something additional and exciting to find. The book will be useful to both casual and serious fern (and orchid) students visiting in and beyond the Mariana Islands, and is recommended to all – D. B. LELLINGER, Dept. of Botany, Smithsonian Institution, Washington, DC 20560.

Monograph of the Neotropical Fern Genus *Stigmatopteris* (Dryopteridaceae) by Robbin C. Moran. 1991. Ann. Missouri Bot. Gard. 78:857–914.

According to this impressive monograph, *Stigmatopteris* contains 24 species distributed primarily in wet montane forests from the Antilles and southern Mexico to southern Brazil. Members of this genus are distinguished from all other dryopteroid genera by pinnae bearing pellucid, punctate glands, by scales with uniseriate cilia ending in bulbous, glandular cells, and by veins which have clavate apices ending well short of pinnae margins. This work provides a taxonomic history of the genus and sections on distribution, morphology, and species relationships. The section on morphology contains concise discussions of characters used to distinguish *Stigmatopteris*. These characters are pubescence, indusia, glands, scales, veins, hairs, sporangia, stipes, and spores. Character states of scales, buds or bulblets, veins, pinnae bases, blade dissection. pubescence, pinnae are used to generate cladograms depicting species relationships. Two new species are described and one new combination is made in this work. One species is possibly a hybrid. Keys to the species utilize characters which are readily available from properly pressed fronds. Each species treatment includes a complete nomenclatural citation, designation of types, synonymy, a thorough description, citation of specimens examined, and a discussion of taxonomy. The author has crafted fine, diagnostic illustrations for each species and clear distribution maps. The work concludes with excluded taxa, names of uncertain application, literature cited, an index to collectors' names, and an index to names. Dr. Moran has compiled a well written monograph of *Stigmatopteris* which could serve as a model for those preparing monographic treatments. This excellent treatise should be in the library of everyone interested in neotropical pteridophytes — W. Carl Taylor, Milwaukee Public Museum, Milwaukee, Wisconsin 53233.

INFORMATION FOR AUTHORS

Authors are encouraged to submit manuscripts pertinent to pteridology for publication in the *American Fern Journal.* Manuscripts should be sent to the Editor. Acceptance of papers for publication depends on merit as judged by two or more referees. Authors are encouraged to contribute toward publishing costs; however, the payment or non-payment of page charges will affect neither the acceptability of manuscripts nor the date of publication.

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Papers longer than 32 printed pages may be sent to the Editor of *Pteridologia* (Memoir Editor, see cover 2)

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QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

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AMERICAN FERN JOURNAL 83(2): 41-46 (1993)

The Promotion of Spore Germination and Gametophyte Development in *Ophioglossum palmatum* by Low pH

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Gametophytes of several terrestrial species of the Ophioglossaceae have been grown from spores in axenic culture (Whittier, 1972, 1981, 1983; Gifford & Brandon, 1978; Whittier & Peterson, 1984). As more species have been included in these investigations, it has become evident that one set of cultural conditions is not effective for all species. For the species examined to date, darkness is needed for spore germination and sugar is necessary for growth of these nonphotosynthetic gametophytes. However, variations in the mineral content, nitrogen source, and temperature are needed to promote or allow gametophyte formation from spores in some species (Whittier, 1981; Melan & Whittier, 1990).

Since variation exists in the requirements for gametophyte development in culture for the terrestrial species, an examination of the conditions necessary for gametophyte development of an epiphytic species was undertaken. It seemed appropriate to determine if there was anything unusual about the conditions that are necessary to obtain gametophytes of epiphytic species from spores. *Ophioglossum palmatum,* an epiphyte native to the United States, was selected for testing. In Florida, this *Ophioglossum* typically grows on cabbage palms (Mesler, 1975), however there is one report of it growing on saw palmettos (Nauman & Moyroud, 1986). The objectives of this study were to determine the requirements for spore germination, the pattern of early gametophyte development, and the possibility of raising mature gametophytes in axenic culture.

MATERIALS AND METHODS

Spores of *Ophioglossum palmatum* L. were obtained from fertile spikes collected in southern Florida. Since *O. palmatum* is an endangered species in Florida, only the fertile spikes were collected and no plants or leaves were removed for voucher specimens. The spores had the typical shape and internal organization for *Ophioglossum* spores and very few atypical spores with irregular shapes or disorganized contents were present in any of the spore collections.

The spores were surface sterilized with 20% Clorox by the technique of Whittier (1973). The surface-sterilized spores were sown in the early experiments on 15 ml of nutrient medium in 20 mm diameter culture tubes, which were tightly closed to reduce moisture loss. Except for a few light controls, the spores were maintained in darkness at 20+1°C for 3 to 12 months. No germination occurred in any of the cultures in the light.

The nutrient medium contained a modified Moore's solution (Moore, 1903). A liter of the basic medium contained 100 mg $MgSO_4 \cdot 7H_2O$, 40 mg $CaCl_2$, 100 mg K_2HPO_4, and 100 mg NH_4Cl, plus FeEDTA and minor elements. The carbon source was 0.2% glucose. The media employed in the early experiments were solidified with 0.6% agar and were pH 5.0 or 5.5 after autoclaving. Other aspects of the nutrient medium and culture

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vessels will be considered with the results since they were important for spore germination and gametophyte growth.

Results

The early experiments had limited success. A low percentage of germination occurred on the pH 5.0 medium after three months. However, the young gametophytes aborted at the 2- or 3-celled stage. Efforts to modify the nutrient medium involved alterations to the pH. A wider pH range was employed with these media and after nine months, there was good germination on the more acidic media (Table 1). The gametophyte growth was again limited and rarely exceeded the 4-celled stage.

After several futile attempts were made to improve the growth of the gametophytes on these media, the natural habitat of the gametophyte was analyzed more closely. In nature these gametophytes grow in the black humus which collects between the petiole bases and trunks of *Sabal palmetto* (Walt.) Lodd. ex Schultes. Mesler (1975) has found that only the humus which remains moist will have gametophytes and Wherry (1964) reported that the pH of this material is subacid. The nutrient media employed would seem to satisfy both conditions. They were moist and at pH 5.0 or 5.5 which fits Wherry's pH scale (1920) as being subacid.

Table 1. Spore germination of *Ophioglossum palmatum*[1]

	pH							
Medium and duration	3.4	4.0	4.2	4.6	4.7	5.0	5.4	6.2
Agar, 9 mo.	-	-	-	48%	-	23%	0%	0%
Agar, 3 mo.	-	16%	5%	-	-	-	-	-
Liquid, 8 mo.	14%	55%*	-	-	48%*	-	20%	-

[1]Percentage of germination followed by an asterisk (*) indicates media which supported gametophyte growth beyond the 4-celled stage.

Poor growth indicated that the nutrient medium did not satisfy all of the requirements of these gametophytes. Since there were some indications that the pH of the humus might be more acidic than assumed, its pH was tested. The humus is strongly acid at pH 4.0+0.1 which suggested that the pH of the nutrient medium should be changed.

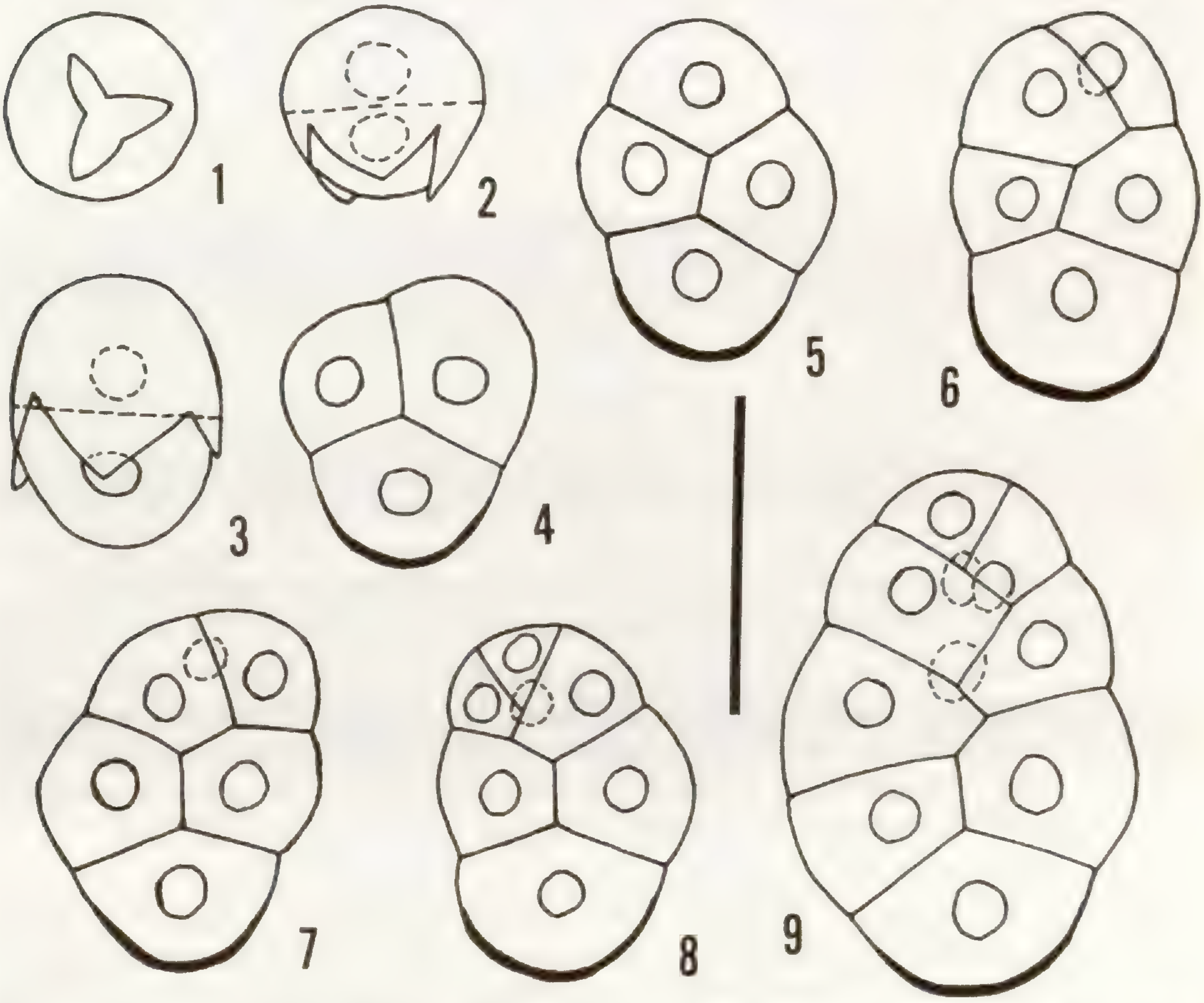
There are difficulties in solidifying agar of very acidic nutrient media. However, increasing the concentration of agar did alleviate the problem to some extent. After three months on a medium with 1.5% agar and at pH 4.0 after autoclaving, 16% of the spores germinated (Table 1). Germination on a medium at pH 4.2 was 5%.

In an effort to better control the initial pH of the nutrient medium, shallow liquid culture in plastic culture flasks was tested. The nutrient media were filter sterilized and had initial pH readings of 3.4, 4.0, 4.7, and 5.4. Spores germinated on all media and the higher percentages of germination, 55% and 48%, occurred on media with pH 4.0 and 4.7 respectively (Table 1). Importantly, the media at pH 4.0 and 4.7 supported gametophyte growth with the best being at pH 4.0.

Cracking open of the triradiate ridge was the first indication that germination had started (Fig. 1). Before there was any significant enlargement of the spore, a division perpendicular to the polar axis of the spore occurred (Fig. 2). It divided the spore equally forming a distal cell (away from the triradiate ridge) and a proximal cell (near the triradiate ridge). The proximal cell enlarged forcing the three lobes of the cracked spore coat apart

and bulged out of the spore coat (Fig. 3). The outer wall of the proximal cell thickened as the gametophyte developed but mucilage did not appear to be secreted through this thick wall (Fig. 4). The second division, which was slightly oblique to the polar axis of the spore, divided the distal cell into two cells with one slightly larger than the other (Fig. 4). The third division, which occurred in the larger of these cells (Fig. 5), was initially perpendicular to the cell wall of the second division. The 4-celled gametophyte was a flat plate of cells. The fourth division occurred in the terminal cell and the gametophyte began to thicken (Fig. 6). Additional divisions in the terminal cell established an apical cell (Fig. 7, 8, 9). Although there was some variability in the stage at which the apical cell was established, the apical cell was usually present by the 8- or 9-celled stage. The proximal cell did not divide during the early stages of gametophyte development. Whether it underwent divisions at later stages was not determined.

Gametophytes were transferred from the pH 4.0 and 4.7 liquid media to new media in an effort to obtain mature gametophytes. Even though these cultures were rather old at the time of transfer and most of the gametophytes were of microscopic size, it was the



Figs. 1–9. Young gametophytes of *Ophioglossum palmatum*. Fig. 1. Opening of triradiate ridge. Fig. 2. Early two-celled gametophyte. Cell wall and nuclei covered by spore coat indicated by dotted lines. Fig. 3. Two-celled gametophyte with proximal cell bulging out of the spore coat. Fig. 4. Three-celled gametophyte with thickened proximal cell wall. Figs. 5–8. Four-, five-, six-, and seven-celled gametophytes respectively. No effort was made to indicate walls of cells positioned behind other cells, however the nuclei of these cells are indicated by dotted lines. Fig. 9. Ten-celled gametophyte with apical cell. Bar = 100 μm.

only source of gametophytes for new cultures. They were transferred to a new medium with 1.0% agar and at pH 4.4 after autoclaving. The microscopic-sized gametophytes were pipeted from the liquid media to the new medium. The macroscopic-sized gametophytes were moved individually with a spatula to the new medium. The microscopic-sized gametophytes ceased to grow after the transfer, however the macroscopic gametophytes grew very slowly on the new medium. They first became cylindrical and later began to branch.

Discussion and Conclusions

Nutrient media with ammonium-nitrogen are very effective for the germination of *Botrychium* spores (Melan & Whittier, 1990). This is also true for spores of *O. palmatum* but the pH of the medium must be lowered to get high percentages of germination. The most germination was obtained on a medium at pH 4.0, although germination did occur on media with a somewhat higher or lower pH.

The initial germination of *O. palmatum* spores occurred at about 3 months in the dark, which is within the range of times necessary for the germination of other spores of the Ophioglossaceae (Whittier, 1981). If left for longer periods on an optimal medium, a majority of the spores germinated (Table 1). No effort was made to determine the highest percentage of germination possible in axenic culture. Since there were very few aborted spores in the spore collections, it would appear that almost all the spores were viable and had the possibility of germinating.

Studies using spores of leptosporangiate ferns have shown that best germination occurs at a slightly acidic or neutral pH (Miller, 1968). However, there is variation in how spores of the leptosporangiate ferns respond to strongly acidic conditions. In some species there is no germination (Hevly, 1963) or a low percentage of germination (Mohr, 1956). With other species moderate levels of germination occur but the gametophyte development is restricted (Courbet, 1955; Otto et al., 1984). Spores of terrestrial species of Ophioglossaceae respond to the pH of the nutrient medium similarly to those of the leptosporangiate ferns (Whittier, 1981). They germinate best at a slightly acidic pH. The spores of *O. palmatum* are unusual in that their germination is best with strongly acidic conditions and is reduced or eliminated at slightly acidic or neutral conditions.

Besides being important for germination, the low pH was critical for the growth of the young gametophyte of *O. palmatum*. Growth beyond the 3- or 4-celled stage did not occur on media at pH 5.0 and higher or at pH 3.4. The media that allowed the gametophytes to develop beyond the 4-celled stage were liquid media at pH 4.0 and 4.7. For whatever reason, gametophyte development, although initiated by spore germination, never proceeded beyond the 4-celled stage on nutrient media solidified with agar.

The development of the young gametophytes of *O. palmatum* agrees with that of *O. vulgatum* in axenic culture (Whittier, 1981). The early pattern of divisions is similar although the gametophytes of *O. vulgatum* become two cells thick at an earlier stage than those of *O. palmatum*. The large cells and somewhat different pattern of cell divisions make the *Ophioglossum* gametophytes distinctive from the young gametophytes of *Botrychium* (Whittier, 1981). The formation of an apical cell by the young gametophytes of *Ophioglossum* also distinguishes these gametophytes from those of *Botrychium* which do not form apical cells.

The development of the gametophyte and its apical meristem from products of the dis-

tal cell is characteristic of the Ophioglossaceae. This pattern is not common among the other pteridophytes. It has only been found in *Actinostachys pennula* for pteridophytes with mycorrhizal gametophytes (Bierhorst, 1975). Spores of leptosporangiate ferns have several germination patterns, however, in only two of these does the proximal cell become a rhizoid and the distal cell develop into the photosynthetic gametophyte (Nayar and Kaur, 1971). Under natural conditions, there may be a possible advantage for the Ophioglossaceae to produce the growing portion of the gametophyte from the distal cell. Campbell (1911) has reported that the mycorrhizal infection of the young gametophytes of *O. pendulum* occurs through the wall of the proximal cell. With this germination pattern the proximal cell, which is first out of the spore coat and becomes a reduced rhizoid, can be infected by a mycorrhizal fungus during the early states of germination without interfering with the meristematic portion of the young gametophyte.

Microscopic multicellular gametophytes failed to grow into cylindrical gametophytes when transferred to an agar medium at pH 4.4. Whether this was a problem with the medium or the age of the young gametophytes has not been resolved at this time. Macroscopic gametophytes which were transferred continued to grow slowly on this medium. Thus, older gametophytes can be grown on a medium solidified with agar. The shape of these gametophytes in culture is sufficient to identify them as *Ophioglossum* gametophytes and their branching is characteristic of gametophytes of *O. palmatum.*

The germination of spores of *O. palmatum* in the dark, which is true for other members of the Ophioglossaceae, and in a strongly acidic environment helps to explain the habitat of these gametophytes in nature. The black humus at the petiole bases of cabbage palms would provide a dark and strongly acidic environment. Moist humus of this type would present the proper conditions for germination. Since gametophyte development in culture was also better at a low pH, humus having a mycorrhizal fungus would satisfy the requirements for gametophyte growth. Although the promotion of gametophyte development by strongly acidic conditions is unusual in ferns, it is apparent that a low pH is important for the development of gametophytes of *Ophioglossum palmatum.*

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The authors thank Robert Kral, Clifton Nauman, John Fillyaw, and Richard Roberts for assistance with early aspects of this project. This study was supported in part by the Vanderbilt University Research Council.

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REVIEW

New Jersey Ferns and Fern-Allies by James D. Montgomery and David E. Fairbrothers. Rutgers University Press, New Brunswick, NJ 08901. ISBN 0-8135-1817-2, Cloth $45.00. 292 pp.

This is an excellent and comprehensive treatment of the 83 species plus 32 hybrid pteridiophytes found in New Jersey. The authors have written a very useful manual. The descriptions are accurate, brief, and clearly written. The keys are carefully crafted to focus on distinctions to fertile specimens and to vegetative specimens. The detailed illustrations of whole fronds and enlargements of diagnostic features, prepared by Kathleen L. John-Alder, are excellent. The treatments include comments on taxonomic status, habitat, chromosome counts, habit of growth, endangered species status, and distribution maps. The size of dot varies, with larger dots indicating older collections, allowing the reader to infer spread or depletion of a species. Access to the book is facilitated with illustrations of technical terminology, with discussions of the biology of hybridization, and with an explanation of the physiographic regions and habitats of New Jersey. The bibliography is extensive, providing ample means to consult the literature. Interestingly, the ferners who collected and investigated ferns in New Jersey over the last 250 years include many eminent botanists, including Bartram, Kalm, Michaux, Pursh, Barton, Nuttall, Torrey, Britton, and Stone. This manual is useful to amateur and expert alike. — JAMES H. PECK, Department of Biology, University of Arkansas at Little Rock, Little Rock, AR 72204.

Photosynthetic Adaptability of Two Fern Species of a Northern Hardwood Forest

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The capacity for acclimation, "the gradual and reversible adjustment of physiology or morphology as a result of changing environmental conditions" (Lincoln, et al., 1982), varies among plant species (Chapin, 1980; Hicks & Chabot, 1985). Fast-growing species of partially shaded habitats have the greatest ability to acclimate to maximize production in shade (Grime, 1981). Shade leaves are generally thinner and have larger leaf areas, lower concentrations on a mass basis and contents on an area basis of RUBISCO and leaf protein, and frequently higher chlorophyll contents but lower chlorophyll concentrations than sun leaves (Björkman & Holmgren, 1963; Ludlow & Wolf, 1975; Boardman, 1977; Hicks & Chabot, 1985). A positive correlation between net photosynthetic rate per unit leaf area and N content has been reported for a variety of plants including woody and herbaceous species (Field & Mooney, 1986; Evans, 1989; Karlsson, 1991; Tuohy, et al., 1991). Higher light-saturated net photosynthetic rates per unit leaf area have been found for sun-grown than shade-grown conspecifics (Björkman & Holmgren, 1963). Seasonal changes in photosynthesis have been reported by Bauer, et al. (1991) for *Dryopteris filix-mas* (L.) Schott.

The present study was undertaken to determine the photosynthetic adaptabilities to sun and shade of two contrasting fern species of a northern hardwood forest. *Dryopteris intermedia* (Muhl. ex Willd.) Gray is a semi-evergreen fern of northern hardwood forest understories in North America, frequently occurring beneath the forest canopy but also found in gaps of recent origin (Siccama, et al., 1970; Hughes & Fahey, 1991). Congeners *(Dryopteris spinulosa* (O.F. Muell.) Watt and *D. marginalis* (L.) Gray) have been described by Sparling (1967) as shade-tolerant. *Dennstaedtia punctilobula* (Michx.) Moore is a summergreen fern frequently occurring in forest gaps and clearings but also found beneath the canopy (Flaccus, 1959; Siccama, et al., 1970; Hughes & Fahey, 1991). We hypothesized that both sun- and shade-grown sporophytes of the competitive, mainly gap-phase species, *Dennstaedtia punctilobula,* would have higher net photosynthetic rates than both sun- and shade-grown sporophytes of the shade-tolerant species, *Dryopteris intermedia.* We also expected that sun-grown sporophytes of each species would have higher net photosynthetic rates and N contents per unit leaf area, but lower N concentrations per unit dry mass than shade-grown sporophytes.

MATERIALS AND METHODS

Fern sporophytes of each species were collected from the Huntington Forest within the Adirondack Park, New York in August 1991. Six plants (presumably genets) of

Table 1. Characteristics (± SE) of *Dryopteris intermedia* and *Dennstaedtia punctilobula* fern fronds. Mean values of 3 samples unless noted otherwise. * indicates significantly different ($P \leq 0.05$) following *t* - test.

Variable	Shade-grown	Sun-grown	*t*	*df*	*P*-value
Dryopteris intermedia					
Area (cm^2)	43.40±2.47	38.33±1.10	-1.88	4	0.13
Dry mass (g)	0.153±0.004	0.173±0.006*	2.72	4	0.05
Dry mass/area (g/cm^2)	0.0035±0.0002	0.0045±0.00006*	4.97	4	0.01
C concentration (% dry mass)	47.61±0.17	47.81±0.18	0.81	4	0.46
C content (mg/cm^2)	168.28±9.33	215.14±1.99*	4.91	4	0.01
N concentration (% dry mass)	2.45±0.15	2.06±0.27	-1.29	4	0.27
N content (mg/cm^2)	8.68±0.83	9.24±1.12	0.40	4	0.71
Dennstaedtia punctilobula					
Area (cm^2)	29.08±3.32	25.08±1.98	-0.82	4	0.46
Dry mass (g)	0.046±0.010	0.075±0.009	2.15	4	0.10
Dry mass/area (g/cm^2)	0.0016±0.0002	0.0029±0.0002*	4.89	4	0.01
C concentration (% dry mass)	46.93±0.04	47.62±0.52[a]	1.03	3	0.38
C content (mg/cm^2)	79.78±9.31	138.30±11.42*	3.59	3	0.04
N concentration (% dry mass)	2.49±0.24	2.25±0.39[a]	-0.45	3	0.68
N content (mg/cm^2)	4.19±0.09	6.62±1.53	1.23	3	0.31

[a] n = 2, limited sample

Dryopteris intermedia were collected from beneath a forest canopy and six plants of *Dennstaedtia punctilobula* were collected from a nearby clearing. The plants were potted in organic soil in which the plants occurred and transferred to a greenhouse.

Three plants of each species were placed in direct sunlight and three plants of each species were placed in shade. The plants were watered to field capacity twice daily by an automated drip-irrigation system. Typical shade and sun irradiance levels were 62 and 945 μmol $m^{-2}s^{-1}$ at solar noon on a clear day and 27 and 92 μmol $m^{-2}s^{-1}$ on a cloudy day, respectively, and compare well with field measurements (Brach, unpublished data). The ferns were allowed to acclimate and grow for two months prior to gas exchange measurements.

Using a steady-state differential gas analysis apparatus, carbon dioxide exchange measurements were made on the most recently developed, fully-expanded frond of each plant that developed during the acclimation period. Since all fronds which developed after transfer were sterile, measurements were made only on sterile fronds. System design and materials followed standard criteria (Bloom, et al., 1980). Conditions chosen and maintained during measurement were a temperature of 25°C, 85% relative humidity, and 350μl l^{-1} CO_2. Light was supplied by a 1.5 kW xenon arc lamp. Photosynthetic photon flux density (PPFD) was increased progressively to five levels by removing neutral density filters.

Following gas exchange measurement of each frond, the portion of the frond that was in the cuvette was excised and its leaf area measured using an area meter (Model LI-3000 with LI-3050A conveyer, Li Cor Inc.). Fronds were dried to constant mass at 65°C. Carbon and N concentrations were determined using a CHN analyzer (Model 240C,

Table 2. Repeated measures ANOVAs for net photosynthetic rate per unit leaf area and per unit dry mass for the two fern species.

Source	*MS*	*df*	*F*	*P*	(H-Fp[a])
Net photosynthetic rate per unit leaf area					
species	24.81	1	3.97	0.0813	
treatment	7.25	1	1.16	0.3125	
spp. x trmt.	9.14	1	1.46	0.2607	
between subject	6.26	8			
PPFD level	0.78	4	8.83		0.0030
spp. x level	0.30	4	3.38		0.0623
trmt. x level	0.24	4	2.72		0.0990
spp. x trmt. x level	0.04	4	0.49		0.6135
within subject	0.08	32			
Net photosynthetic rate per unit dry mass					
species	402685.95	1	27.53	0.0008	
treatment	223260.00	1	15.26	0.0045	
spp. x trmt.	161160.20	1	11.02	0.0106	
between subject	14624.34	8			
PPFD level	1289.21	4	10.57		0.0018
spp. x level	721.59	4	5.92		0.0150
trmt. x level	246.54	4	2.02		0.1704
spp. x trmt. x level	122.85	4	1.01		0.3818
within subject	121.63	32			

[a] Huynh – Feldt corrected level of significance

Perkin-Elmer) and contents calculated. Two analytical replicates were averaged per sample.

For each species, Student's *t*-tests (SAS, 1985) for each species were used to determine if leaf area, dry mass, dry mass per unit leaf area; C and N concentrations and contents were significantly different between growing conditions. Net photosynthetic rates of both species per unit leaf area and per unit dry mass were analyzed by repeated measures ANOVAs (SAS, 1985; Potvin & Charest, 1991). The main effects tested were species, treatment (growing conditions), and PPFD level. Species, treatment and species X treatment were considered as between-subject effects, while PPFD level was considered a within-subject factor.

Results

Morphological characteristics of the ferns grown under contrasting greenhouse conditions are presented in Table 1. In both species, sun-grown plants had significantly higher dry mass per unit leaf area than shade-grown plants. Sun leaves of sun-grown ferns of both species were yellow-green as compared to darker green shade leaves of shade-grown ferns.

Fronds of shade-grown ferns had generally higher, but not significantly different ($P > 0.05$), N concentrations and generally lower, but not significantly different ($P > 0.05$), N contents compared to fronds of sun-grown ferns. Fronds of sun-grown ferns had significantly higher C contents than fronds of shade-grown ferns (Table 1).

Dennstaedtia punctilobula had higher net photosynthetic rates per unit dry mass than *Dryopteris intermedia* (Table 2, Fig. 1), but not per unit leaf area (Table 2, Fig. 2). Shade-grown *Dennstaedtia punctilobula* had significantly higher net photosynthetic rates per

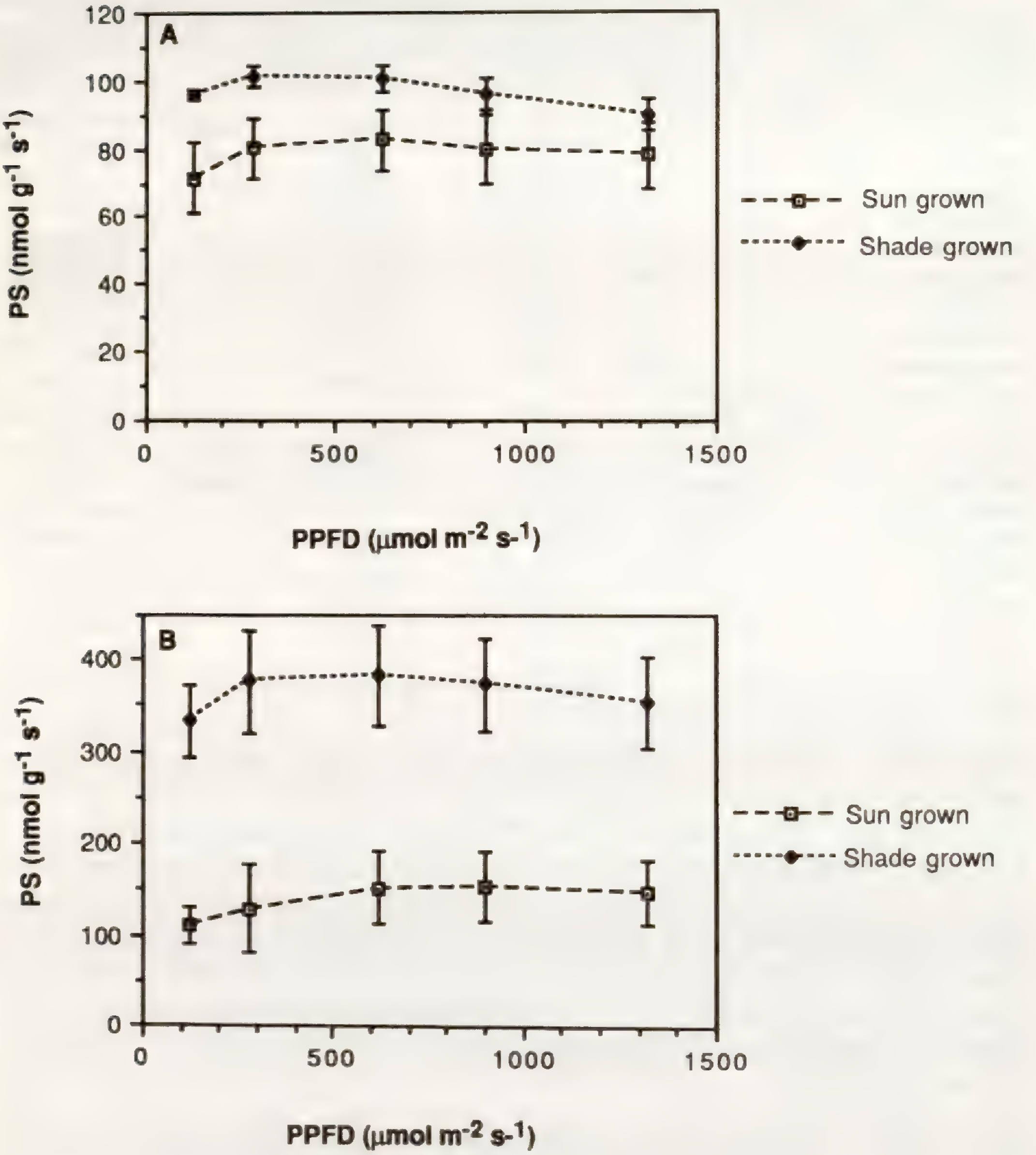

Fig. 1. Mean net photosynthetic rate (PS) (± SE) per unit dry mass of A) *Dryopteris intermedia* and B) *Dennstaedtia punctilobula* for five levels of photon flux density (PPFD). Marks represent mean values of 3 samples.

unit dry mass than sun-grown conspecifics over all PPFD levels used (Fig. 1b). Net photosynthetic rates per unit leaf dry mass were significantly affected by species, treatment, species X treatment, PPFD level, and species X PPFD level (Table 2). Species, treatment, and the species X treatment interaction accounted for 50, 28, and 20% of the total variance, respectively. Additionally, shade-grown *Dennstaedtia punctilobula* had higher net photosynthetic rates per unit leaf area than sun-grown conspecifics at the lowest PPFD level used (Fig. 2b).

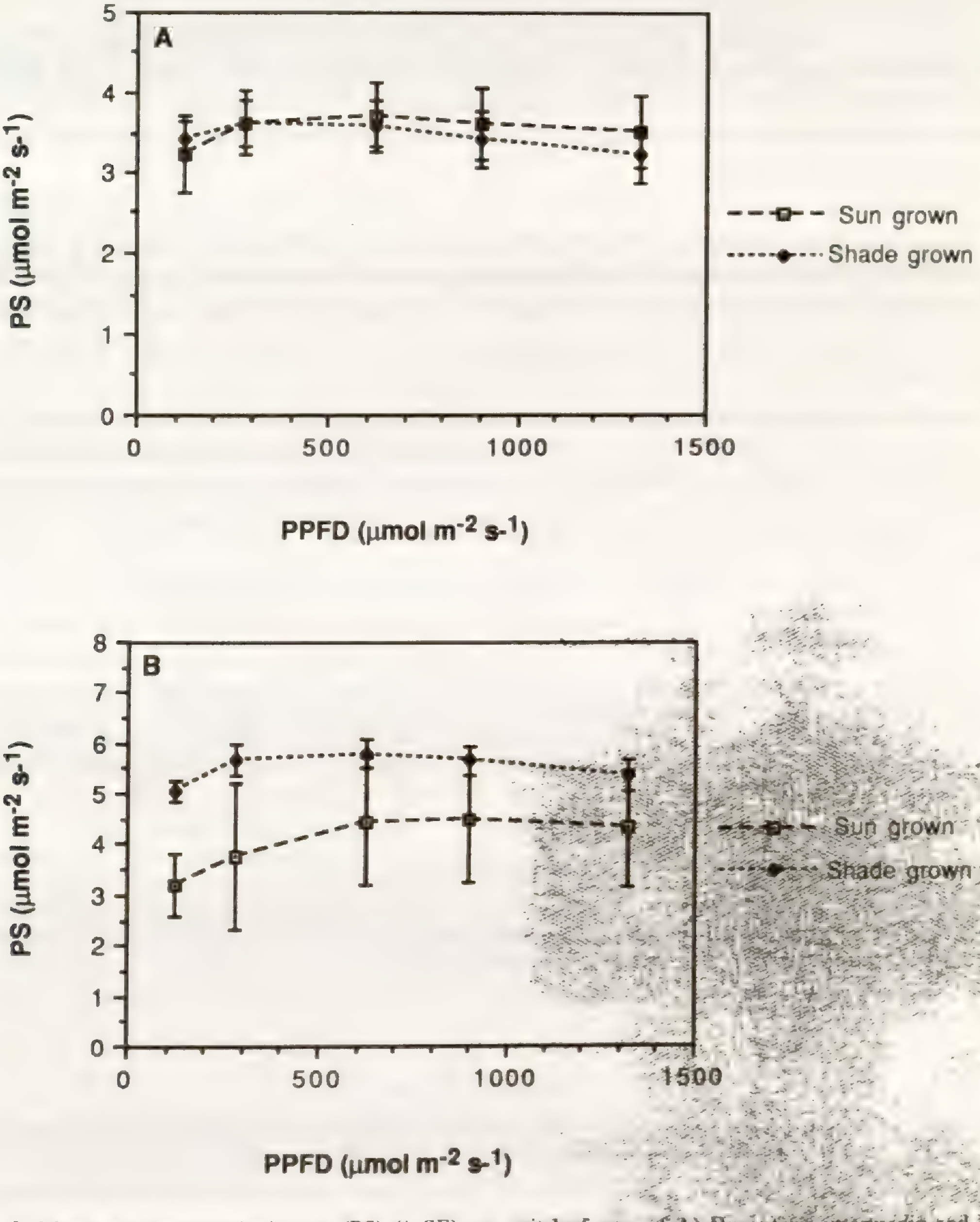

Fig. 2. Mean net photosynthetic rate (PS) (± SE) per unit leaf area of A) *Dryopteris intermedia* and B) *Dennstaedtia punctilobula* for five levels of photon flux density (PPFD). Marks represent mean values of 3 samples.

Discussion

Shade-grown *Dennstaedtia punctilobula* had higher net photosynthetic rates per unit dry mass than *Dryopteris intermedia*. However, contrary to our expectations, shade-grown *Dennstaedtia punctilobula* had higher net photosynthetic rates than sun-grown conspecifics. Plausible reasons for this response include adaptability of shade leaves to utilize periodic sunflecks (Chazdon & Pearcy, 1991; Gildner & Larson, 1992a), higher chlorophyll contents in shade leaves than sun leaves (Boardman, 1977), and photoin-

hibitory and chloroplast damage in sun leaves (Björkman & Holmgren, 1963).

Both species developed phenotypic sun and shade leaves in response to experimental sun and shade conditions. Higher C contents in fronds of sun-grown ferns reflect higher dry mass per unit leaf area of sun-grown fern fronds. Nutrient stress in sun leaves is unlikely since frond N concentrations were similar to those determined for field plants (Brach, unpublished data).

The lack of acclimation in *Dryopteris intermedia* is similar to that of understory species of densely shaded tropical and temperate forests (Björkman, et al. 1972; Gildner & Larson, 1992b). As proposed for other species of shaded habitats, *Dryopteris intermedia* may have evolved semi-evergreen leaves in response to low water and nutrient availability (Sobrado, 1986; 1991) and for photosynthesis when the canopy is absent or reduced in winter.

Maximum net photosynthetic rates of these ferns were higher than those reported for ferns by Ludlow & Wolf (1975) of 0.7–1.3 μmol $m^{-2}s^{-1}$ and for *Polypodium virginianum* L. by Gildner & Larson (1992b) of 1.2–2.5 μmol $m^{-2}s^{-1}$, and were lower than those reported for a congeneric species, *Dryopteris filix-mas* (L.) Schott of 8–9 μmol $m^{-2}s^{-1}$ by Bauer, et al. (1991).

This study adds to evidence of successional differences in photosynthesis (Bazzaz & Carlson, 1982; Koike, 1988). Shade-grown *Dennstaedtia punctilobula,* a vigorous gap colonizer species, had a higher net photosynthetic rate per unit leaf dry mass than *Dryopteris intermedia,* a shade-tolerant understory species. Higher net photosynthetic rates of *Dennstaedtia punctilobula* grown in shade may allow this species to efficiently utilize low light when beneath the forest canopy and then to colonize canopy gaps as they become available.

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The Influence of Soil Topography and Spore-Rain Density on Gender Expression in Gametophyte Populations of The Homosporous Fern *Aspidotis densa*

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The gametophytes of homosporous pteridophytes, being free-living and independent of the sporophyte, experience an immediacy with the external environment unique among the gametophytes of vascular plants. As a result, the environment directly influences the demographics of homosporous fern gametophytes, particularly their distribution, development, and mating systems. Because most homosporous fern gametophytes are photosynthetic, require light to germinate, and develop gametangia sequentially, environmental factors that alter the quantity and quality of light to neighboring spores and gametophytes may stimulate asynchronous germination, growth, and sexual maturation. The influence of environmental factors upon the population biology of fern gametophytes may be augmented by the production of antheridiogens. The meristematic gametophytes of some species have been shown to produce antheridiogens or as yet unspecified metabolites that generate "antheridiogen effects" (induced germination in darkness, precocious maleness, and delayed development of a notch-meristem) in ameristic neighbors (Näf, 1963, 1975; Schedlbauer & Klekowski, 1972; Hamilton, 1989; Haufler & Gastony, 1989; Schneller, et al., 1990). In some species, individuals with notch-meristems are insensitive to antheridiogen (Näf, 1963). In such species, asynchronous growth and development among neighbors is essential for an antheridiogen to influence the gender composition of a population.

Microvariation in soil topography may alter the availability of light to spores and their ensuing gametophytes. Spores shed on well-illuminated regions of a heterogeneous soil profile are likely to germinate early compared to less-illuminated neighbors. The resulting gametophytes will mature rapidly, and through the action of an antheridiogen, influence the germination and sexual expression of neighbors in poorly illuminated regions.

Because antheridiogens are water soluble and of uncertain stability in the environment (Näf, 1963), and because homosporous fern gametophytes require water for fertilization, the proximity of neighboring gametophytes may be another important determinant of gender composition and mating systems. For example, the probability that colonizing gametophytes are reproductively isolated is associated with a higher probability of selfing, resulting in genetically homozygous progeny without genetic load (Lloyd 1974a, 1974b; Crist & Farrar, 1983; McCauley, et al., 1985; Soltis, et al., 1988; Peck, et al., 1990; Holsinger, 1991; Watano & Masuyama, 1991). Alternatively, in taxa producing dense gametophyte populations, close proximity may facilitate interactions between neighbors, leading to outcrossing mating systems, high levels of genetic load, and heterozygous sporophytes (Lloyd, 1974a, 1974b, 1988; Singh & Roy, 1977; Duckett & Duckett, 1980; Soltis & Soltis, 1986, 1987; Soltis, et al., 1988; Holsinger, 1991). Thus, by decreasing distances between neighbors, dense spore-rain may amplify the influences that asynchronous germination and development have on the sexual expression and mating system of a population and low densities of spore-rain may result in distances too large for interactions among gametophytes (Cousens & Horner, 1970; Tryon & Vitale,

1977; Cousens, 1979; Von Aderkas, 1983; Rubin, et al., 1985; Klekowski & Lloyd, 1968; Lloyd, 1988; Schneller, 1988; Peck, et al., 1990; Hamilton & Lloyd, 1992). This study was designed to determine the influence of soil topography and spore-rain density on the gender composition of gametophyte populations of *A. densa* (Brack. in Wilkes) Lellinger.

A. densa is an endemic of serpentine rock outcrops of western North America. Its range extends from British Columbia south to California and east to Montana and Utah (Wagner, 1957; Smith, 1975; Lellinger, 1985). Gametophytic ontogeny is regulated by an antheridiogen system (Greer, 1991). In isolation, gametophytes develop into meristematic, archegoniate individuals that remain unisexual for more than six months. Antheridia occur only on ameristic gametophytes in multispore cultures or on gametophytes grown on agar that supported a previous generation of meristematic gametophytes. Insensitivity to antheridiogen correlates with the development of a notch-meristem. The antheridiogen of *A. densa* also induces spore germination in darkness.

Methods and Materials

Fertile fronds were collected from five sporophytes at each of the three following California populations of *A. densa:*

1. Alpine lake, Marin Co., south face of Azalea Hill below water tank summit on Bolinas-Fairfax road. T1N, R7W.
2. Horse Mountain, Humboldt Co., south facing slope along summit road; 1/4 mile southeast beyond radio tower. T6N, R4E, S28.
3. Bridge over north fork of Feather River, 1/4 mile south of Pulga city, State Hwy 70, Butte Co., serpentine outcrop above southeast end of bridge. T22N, R5E, S6.

To investigate the influences of soil topography and spore-rain density on the gender composition of gametophyte populations, two soil textures, "fine" and "coarse", were each combined with two spore-rain densities, "low" and "high", to produce a 2 X 2 set of treatments (Table 1). Each treatment combination was replicated across fifteen sporophytes. Gametophyte populations were established, cultured, and observed for gender composition as described below.

Serpentine soil (from the top 5 inches of the soil horizon) was collected from Horse Mtn., Humboldt Co.; and Kneeland airport, Humboldt Co., – both sites of vigorous *A. densa* populations. A 50:50 mixture, by volume, of the two soils was prepared, and the composite sieved into the following size classes: 1) 4.0mm < X < 15.6mm, 2) 1.0mm < X < 4.0mm, and 3) X < 1.0mm. The fine-grained soil was prepared using equal proportions, by weight, of soil classes 2 and 3. The coarse-grained soil was prepared by mixing soil classes 1 and 3 at a weight ratio of 87:13 respectively. Each soil sample was wetted with distilled water, transferred into sterile 100mm x 20mm glass petri dishes until approximately 1/2 full, and autoclaved.

Spores from each sporophyte were sifted to remove sporangia and debris, and allotments were weighed. Low density treatments averaged 0.0073g (± 0.0005g) per replicate, and high density treatments averaged 0.0638g (± 0.0005g) per replicate. To create an even distribution of spores, the spores for each treatment were placed in two 100mm x 20mm glass petri dish lids, and randomized by pouring the spores into one lid, overturning, and rotating while tapping above the other. The procedure was repeated until the spore counts among each of 5 random fields of view in a Wild M5A stereomicroscope deviated by no more than 20 percent. Each spore-rain density treatment was then inoculated on to each soil texture treatment by overturning and tapping the lid.

Table 1. Pooled data (mean ± SD) for gametophyte density and gender for fine vs. coarse soil texture and low vs. high spore-rain density treatments.

Soil Texture/ Spore-Rain Density	Gametophytes/ 1.25cm²	Gender			
		Male	Female	Bisexual	Vegetative
Fine/Low	46.56 (±41.5)	171 (14.3%)	540 (45.2%)	11 (1%)	472 (39.5)
Fine/High	215.3 (±149.3)	716 (45.2)	401 (25.3%)	0 -	468 (29.5%)
Coarse/Low	33.2 (±24.5)	312 (23.3%)	587 (43.8%)	2 (<0.01%)	441 (32.9%)
Coarse/High	370.8 (±291.5)	941 58.7%)	291 (18.1%)	1 (<0.01%)	372 (23.2%)
Fine	129.9 (±140.3)	887 (32.3%)	911 (33.1%)	11 (<0.01%)	940 (34.2%)
Coarse	211.56 (±271.1)	1399 (45.3)	878 (28.4%)	1 (<0.01%)	813 (26.3%)
F = 3.83, p = 0.055					
Low	33.8 (±26.4)	485 (19.1%)	1127 (44.4%)	13 (<0.01%)	913 (36.%)
High	305.6 (±249.4)	1657 (52.4%)	662 (21%)	1 (<0.01%)	840 (26.6%)
F = 32.13, p = 0.000					

All treatments were placed in clear plastic bins lined with moist paper towel to prevent desiccation. Each was maintained in a culture chamber at a constant temperature of 21°C (± 2°C), and illuminated at an irradiance of approximately 60 microeinsteins/sec/cm² under a 12/12 hour light/dark regimen using equal banks of warm and cool white 40 watt fluorescent lights.

Each treatment dish was observed regularly using a Wild M5A stereomicroscope, and patterns of germination, relative size, and morphology noted. After 12 weeks of culture, 5 random 12.5mm² samples were removed from each treatment dish, the gametophytes washed free of debris, separated, and counted at 25x and 50x using a Wild M5A stereomicroscope. A minimum of one hundred gametophytes were randomly selected, observed at 40x and/or 100x using a compound light microscope, and scored for morphology and gender expression.

A two-way Analysis of Variance (Sokal & Rohlf, 1981) was used to analyze the relationship between each treatment (soil texture and spore-rain density) and the percentage of males in the population.

Results

Gametophyte density corresponded with spore-rain density. Mean gametophyte density for populations established by low spore-rain density was 33.8 (±26.4) gametophytes per 1.25cm², and 305.6 (±249.4) gametophytes per 1.25cm² for populations established by high spore-rain density. Determination of gametophyte density in populations established by high spore-rain density was complicated by profuse branching of filamentous and spatulate gametophytes. Consequently, estimates of gametophyte density in these populations are considered crude approximations.

The frequency of males in gametophyte populations of *A. densa* increased significantly under the influences of coarse-grained soil (45.3% male; F = 3.83, p = 0.055), and high spore-rain density (52.4% male; F = 32.13, p = 0.000) (Table 1). The fewest males

(14.3%) occurred in the paired treatments of low spore rain density and fine-grained soil. Germination and sexual maturation of gametophytes appeared to be nearly synchronous, resulting in predominantly female populations. The greatest number of males (58.7%) occurred in the pairwise treatments of high spore-rain density and coarse-grained soil (Table 1). Germination and sexual maturation of gametophytes in populations established by high spore-rain and/or on coarse-grained soil appeared to be asynchronous. Gametophytes on the upper 1/2 of the soil profile were well-illuminated, first to mature, and predominantly female, whereas those in less-illuminated sites were smaller (often filamentous) and predominantly male.

Discussion

The bigametophytic ontogeny and antheridiogen system of *A. densa* established an obligate cross-fertilizing mating system that virtually precludes intragametophytic selfing (Greer, 1991). Because gametophytes with notch-meristems both produce, and are themselves insensitive to, antheridiogen, asynchronous development among neighbors is essential for the establishment of populations of mixed gender. Thus, factors that promote asynchronous germination and development are important determinants of gender composition, fertilization success, and mate competition in populations of *A. densa.*

Both spore-rain density and soil topography influence asynchronous development and the gender composition of gametophyte populations of *A. densa.* I hypothesize that the heterogeneous topography imparted by coarse-grained soil partitions the availability of light to disseminated spores, providing a basis for asynchronous germination, growth, and development. Spores in well-illuminated sites germinate early. The ensuing gametophytes develop rapidly and are the first to reach sexual maturity. Spores in less-illuminated sites fail to germinate or germinate late, and the ensuing gametophytes develop slowly. As a result, their sensitivity to antheridiogen from better illuminated, early maturing neighbors is prolonged.

Increasing spore-rain densities may facilitate the action of antheridiogen by reducing distances between neighbors (neighborhood size). The difference between the mean neighborhood size of low density populations (4.62 sq.mm) and high density populations (0.51sq.mm) was only 4.11sq.mm, suggesting that antheridiogens may influence the germination, growth, and gender of neighbors only within very limited distances. Although the horizontal diffusion of antheridiogen within agar may be considerable (Voeller, 1969), horizontal diffusion within soil may be greatly limited by factors such as extremes in soil moisture content and the gravitational movement of soil water. Furthermore, the stability of antheridiogen within soil is completely unknown. Neighborhood size may be particularly important among gametophytes receiving equivalent illumination, where the influence of antheridiogen may also be restricted by the nearly synchronous development of neighbors.

If antheridiogen activity occurs only within very limited distances, fertilization success will be most likely, and male mate-competition most severe, in *A. densa* populations established on sites with heterogeneous microtopography, and/or by moderate to high densities of spore-rain (as might be expected near the spore source or in sites favored by wind deposition). Conversely, fertilization success may be severely limited and female mate-competition most severe in populations established on sites with little microsite relief, and/or by low densities of spore-rain. In populations established by very low densities of spore-rain, which can occur within a few meters from the source (Peck, et al.,

1990), spore longevity and the accumulation of spore banks would be particularly important factors determining fertilization success.

Factors such as soil topography and spore-rain density that promote asynchronous germination, growth, and development among neighboring gametophytes may indirectly influence the survival of sporophytic progeny. The earliest maturing gametophytes, such as those growing in well-illuminated sites, are likely to produce the first progeny of the reproductive season. The timing of reproduction during the growing season may be one of the most important events predicting progeny survival. This could be particularly true in dense gametophyte populations, where large numbers of progeny are likely to compete for limited space. Progeny produced earliest in the growing season would have distinct competitive advantage in resource capture and establishment. Moreover, progeny produced early in the growing season, regardless of cohort densities, may be more likely to develop to stress tolerant stages before the onset of "nongrowing" seasons. As a fern endemic to serpentine outcrops, where safe sites appropriate for the gametophyte and juvenile sporophyte are greatly restricted, unevenly distributed, and heterogeneous, the gender composition, mate-competition, fertilization success, and progeny survival of *A. densa* populations should vary considerably from site to site.

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Plantlet Formation in Tropical Montane Ferns: A Preliminary Investigation

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Ferns exhibit a variety of reproductive mechanisms. Sexual reproduction and gametophytic apomixis occur via spores, which are dispersed and germinate to form gametophytes. Many ferns also reproduce vegetatively, producing new ramets through rhizomatous growth, fragmentation and reestablishment, and the formation of plantlets. These ramets are genetically identical to the parent sporophyte and are morphologically complex prior to separation from the parent sporophyte. Genera known to produce viviparous plantlets on sporophyte fronds include *Adiantum, Asplenium, Camptosorus, Cystopteris, Diplazium, Tectaria, & Woodwardia* (Page 1979); plants that grow in sheltered habitats in moist forests are the most likely to produce plantlets. Page (1979) notes that vivipary is common in mid-mountain forests; in this study we compare two such forests. The objectives of our study were to survey the incidence of plantlet production in ferns of two tropical montane forests in Costa Rica, and to determine if plantlet production is more common in the higher, wetter forest than the lower, dryer one.

METHODS

We compared two sites in the vicinity of the Monteverde Cloud Forest Reserve (Puntarenas Province, Costa Rica). The Campbell's Woods site is classified as Premontane/Lower Montane Forest Transition (Holdridge, 1967) and is lower (1460 – 1500 m) and dryer than the Nuboso Trail Woods (1520 – 1560m), which are classified as Lower Montane Forest (true "Cloud Forest"). We sampled four 3x3 m plots in each forest, recording for each individual fern sporophyte encountered: species, reproductive status, number of fronds, and cover (area for terrestrial ferns, vertical height for epiphytes). Juveniles were not included.

We defined the various measures of frequency and abundance as follows: Density (D) = the number of individuals per area sampled; relative density (RD) = density of the species divided by the total D for all individuals times 100; frequency (F) = number of plots in which the species occurred divided by the total number of plots sampled; relative frequency (RF) = frequency of the species divided by the total of the F values for all species times 100; and importance (I) = the sum of RD and RF divided by 2. We calculated these for each species at each site.

Fieldwork was done in May 1988. Voucher specimens were determined at the herbarium of the Museo Nacional de Costa Rica by Mike Grayum and Suzanne Koptur, and remain in Koptur's collection in the FIU herbarium.

RESULTS

We encountered in total 32 species of ferns in 15 genera (Table 1, Table 2). Six of these species were observed to produce plantlets. Although many species of *Asplenium* are known to make plantlets, we only observed this phenomenon in *A. harpeodes* and *A.*

Table 1. Fern species in lower montane wet forest, Campbell's Woods: Relative density (RD), relative frequency (RF), importance (I), and reproduction (R).

Species	RD	RF	I	R
Asplenium auritum Sw.	2.63	4.55	3.59	none
A. barbaense Hieron	10.52	4.55	7.53	spores
A. gomezianum Lellinger	5.27	4.55	4.91	spores
A. pteropus Kaulf.	2.63	4.55	3.59	spores
Blechnum ensiforme (Liebm.) C.Chr.	2.63	4.55	3.59	none
Campyloneurum sphenodes (Kunze ex. Kl. Fee)	5.27	4.55	4.91	none
Ctenitis atrogrisea (C. Chr.) Ching	2.63	4.55	3.59	spores
C. hemsleyana (Baker) Copeland	2.63	4.55	3.59	spores
C. subincisa (Willd.) Ching	2.63	4.55	3.59	spores
Diplazium urticifolium Christ	2.63	4.55	3.59	plantlets
Lomariopsis latiuscula (Maxon) Holtt.	15.79	18.18	16.98	spores
Polybotrya osmundacea H. & B. ex Willd.	10.52	4.55	7.53	none
Selaginella anceps Presl.	2.63	4.55	3.59	none
Thelypteris hatchii A. R. Smith	2.63	4.55	3.59	spores
Trichomanes radicans Sw.	7.89	9.09	8.49	spores
T. reptans Sw.	21.04	13.64	17.34	spores

Table 2. Fern species in lower montane rain forest, Nuboso Woods: Relative density, relative frequency, importance, and reproduction.

Species	RD	RF	I	R
Asplenium cirrhatum L. C. Rich ex Willd.	1.06	2.22	1.64	spores
A. gomezianum Lellinger	1.06	2.22	1.64	spores
A. harpeodes Kunze	3.19	4.44	3.82	both
A. maxonii Lellinger	9.58	6.67	8.13	both
A. pteropus Kaulf.	17.02	6.67	11.85	spores
Bolbitis oligarchia (Baker) Hennipman	2.13	2.22	2.18	both
Camplyloneurum sphenodes (Kunze) Fee	4.26	6.67	5.47	spores
Ctenitis atrogrisea (C. Chr.) Ching	1.06	2.22	1.64	spores
C. hemsleyana (Baker) Copeland	4.26	6.67	5.47	spores
Cyathea fulva (Mart. & Gal.) Fee	1.06	2.22	1.64	none
Diplazium cristatum (Desr.) Alston	1.06	2.22	1.64	spores
D. lehmanii Hieron	1.06	2.22	1.64	plantlets
D. multigemmatum Lellinger	3.19	2.22	2.71	both
D. urticifolium Christ	17.02	6.67	11.85	plantlets
D. werckleanum Christ	2.13	4.44	3.29	none
Elaphoglossum eximium (Mett.) Christ	1.06	2.22	1.64	spores
E. latifolium (Sw.) J. Sm.	1.06	2.22	1.64	spores
Grammitis taxifolia (L.) Proctor	1.06	2.22	1.64	spores
Hymenophyllum consanguineum Morton	1.06	2.22	1.64	spores
Lomariopsis latiuscula (Maxon) Holtt.	7.45	8.89	8.17	spores
Pecluma pectinata (L.) Proctor	1.06	2.22	1.64	spores
Peltapteris peltata (Swartz) Morton	3.19	4.44	3.82	spores
Polypodium fraxinifolium Jacq.	1.06	2.22	1.64	none
Thelypteris hatchii A. R. Smith	3.19	4.44	3.82	spores
Trichomanes reptans Sw.	10.64	8.89	9.77	spores

Fig. 1. Plantlet production from leaf tips in *Asplenium maxonii*. The plantlet is not shaded in the drawing. The bar scale is 2 cm.

maxonii; in both species, elongated leaf-tips produced plantlets (Fig. 1). Three of the four *Diplazium* species we encountered were observed with plantlets *(D. lehmanii, D. multigemmatum,* and *D. urticifolium);* all of these develop buds on the abaxial surface of the frond at the intersection of a major vein and the midrib, the plantlet developing first roots, then leaves that curve around to the adaxial leaf surface (Fig. 2). The unusual *Bolbitis oligarchia* has dimorphic fronds and trifoliate leaves, and plantlets are formed in the middle of the 3 leaflets on the adaxial surface of the trophophylls (leaves not specialized for spore production) (Fig. 3). We also observed plantlets on *Lomariopsis latiuscula,* but not on any individuals in our study plots.

We found 16 species in 10 genera of ferns in plots sampled in the lower elevation wet forest site, Campbells Woods (Table 1). Ten species were found sporing, and only one species, *Diplazium urticifolium,* had plantlets at the time of our census. The species with the greater importance values reproduced by spores alone.

Table 3 – Comparative importance of fern species occurring at both sites. I = importance, C = Campbell's Woods, N = Nuboso Woods.

	I (C)	I (N)	Reproduction
Asplenium gomezianum	4.91	1.64	spores
A. pteropus	3.59	11.85	spores
Campyloneurum sphenodes	4.91	5.47	spores
Ctenitis atrogrisea	3.59	1.64	spores
C. hemsleyana	3.59	5.47	spores
Diplazium urticifolium	3.59	11.85	plantlets
Lomariopsis latiuscula	16.98	8.17	spores
Thelypteris hatchii	3.59	3.82	spores
Trichomanes reptans	17.34	9.77	spores

Of 25 species in 15 genera found in the higher elevation rain forest site (Nuboso), 20 species were making spores, and 6 species were making plantlets (Table 2). Four species had both spores and plantlets, 2 species had plantlets only. Of the species with the greater importance values, two *(Asplenium maxonii* and *Diplazium urticifolium)* produced plantlets; other plantlet producers had relatively low importance values.

Nine species occurred in plots at both sites (Table 3). Only one species of fern was found bearing plantlets in both forests *(Diplazium urticifolium).*

The properties of the fern species at the two sites are compared in Table 4. There were more species at the higher elevation site, and a larger proportion of species producing plantlets (24% vs. 6%). A larger proportion of ferns from the Nuboso site were sporulating (80% vs. 63%), and 16% had both plantlets and spores. A larger proportion of ferns in Campbells Woods were non-reproductive (31% vs. 12%).

Similar comparisons done on an individual plant basis are less dramatic (Table 5). For plantlet production on individuals, Fisher's exact test (2-tailed) is significant (p = .024); 18% of individuals in Nuboso Woods produce plantlets, versus only 3% in Campbells Woods. Spore production does not differ significantly between sites; 32% of individuals in Nubosos Woods versus 45% in Campbells Woods.

Fig. 2. Plantlet production on fronds in *Diplazium urticifolium.* From left to right: tiny developing frond of plantlet on abaxial surface of parent sporophyte frond; plantlet grows, with 4 fronds; two plantlets on old frond ravaged by time and herbivores; plantlet rooted in soil. The plantlets are not shaded in the drawing. The bar scale is 2 cm.

Fig. 3. Plantlet production in center of frond of *Bolbitis oligarchia*. The plantlet is not shaded in the drawing. The bar scale is 2 cm.

Table 4 – Reproductive status of fern species encountered in plots (4 replicates in each forest type).

	$\bar{x}$ + s.d.		Total (Proportion)	
	Nuboso	Campbells	Nuboso	Campbells
Total species	11.5 + 2.9	5.5 + 1.3	25 (1.0)	16 (1.0)
Epiphytic with spores	6.5 + 1.3	2.8 + 9.6	20 (.80)	10 (.63)
with plantlets	2.3 + 1.7	.3 + .5	6 (.24)	1 (.06)
with both	1.0 + 1.1	0	4 (.16)	0 (0)
Non-reproductive	9.0 + 7.5	3.3 + 1.0	3 (.12)	5 (.31)

Table 5 – Reproductive status of individual fern plants encountered in plots (4 replicates in each forest type).

	$\bar{x}$ + s.d.		Total (Proportion)	
	Nuboso	Campbells	Nuboso	Campbells
Total individuals	26.2 + 6.7	10.5 + 1.3	94 (1.0)	38 (1.0)
with spores	7.5 + 1.3	4.3 + 2.6	30 (.32)	17 (.45)
with plantlets	4.2 + 4.0	0.3 + 0.5	17 (.18)	1 (.03)
with both	1.0 + 1.1	0	4 (.04)	0 (0)
Non-reproductive	14.2 + 10.4	5.5 + 2.9	57 (.60)	22 (.58)

Discussion

Our study revealed plantlet production to be more common in a wetter, higher elevation tropical montane forest than in a lower, dryer montane forest. It has been reported that plantlet production under cultivation conditions increases with very high humidity (Farrar, 1968; Mickel, 1976). One could therefore expect that for a fern species occurring over a range of humidity, one might observe more plantlet production in the wetter parts of the range. In East Africa, plantlet production occurs in at least 30% of species (terrestrial & epiphytic) but is associated with the epiphytic habit (Faden, 1973). There was a substantial proportion of epiphytic species in both of the forests we studied, but only in the higher, wetter site did any of the epiphytic species produce plantlets at the time we sampled.

The production of plantlets may be selectively advantageous in areas where there is a continual (not necessarily high) level of disturbance, allowing the offspring to rapidly colonize exposed ground (the terrestrial species); tropical montane areas have landslides and treefalls that provide regular but unpredictable disturbance. A large sporophyte (plantlet) may be able to compete more successfully with other potential colonists than a tiny gametophyte, and would not have immediate need for free water for fertilization.

Plantlet production allows for continuation of the genotype, even if the sporophyte is ravaged by time and enemies. It is not unusual to see nearly moribund fronds of *Diplazium urticifolium,* having lost much of their leaf area to herbivores, attached to big, healthy plantlets rooted in the soil (Fig. 2). In a wet habitat, herbivore damage to a leaf may be additionally hazardous by allowing access to phytopathogens; while the disease may spread within the sporophyte plant, the plantlet may escape contamination and survive to perennate the individual.

The vegetative propagule (plantlet) must regenerate both roots and shoots, though in different ferns the propagule is derived from different organs: in *Nephrolepis,* from the stem; in *Platycerium,* from the root (Richards, et al., 1983); and in *Diplazium* (personal

observation) and *Asplenium* (Mickel, 1976), from the leaves. The production of propagules on stolons permits dispersion of the propagule some distance from the parent sporophyte (Richards, et al., 1983); plantlets produced at or near the tips of long fronds can experience similar distancing.

Our next step is to examine the incidence of plantlet production in certain species of ferns throughout their range, and to monitor the phenology of asexual and sexual reproduction throughout the year. The number of plantlets produced and their fate should also be compared among species and habitats. This will give insight into the edaphic and climatic factors that influence the reproductive strategy. Many of the genera and some of the same species encountered in our mid-elevation study also occur in lowland wet forest at Finca La Selva (Grayum & Churchill, 1987) and will provide an essential and interesting comparison.

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Isoëtes hawaiiensis: A Previously Undescribed Quillwort from Hawaii

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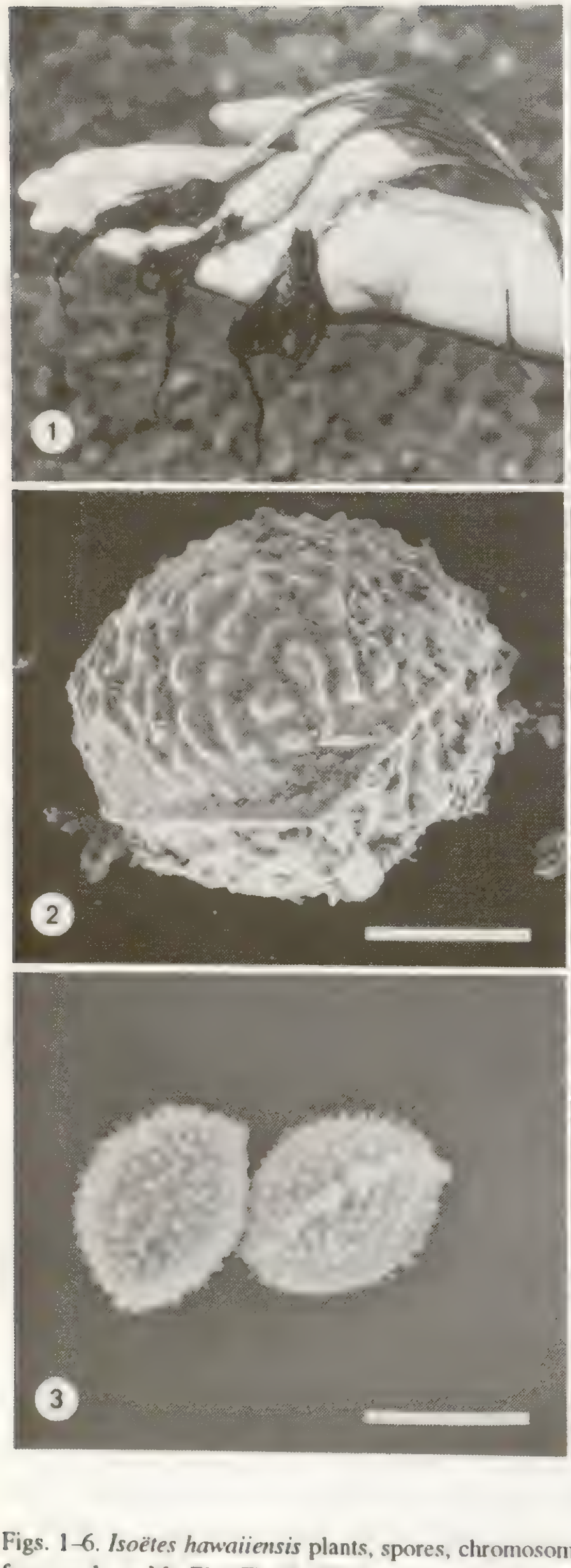
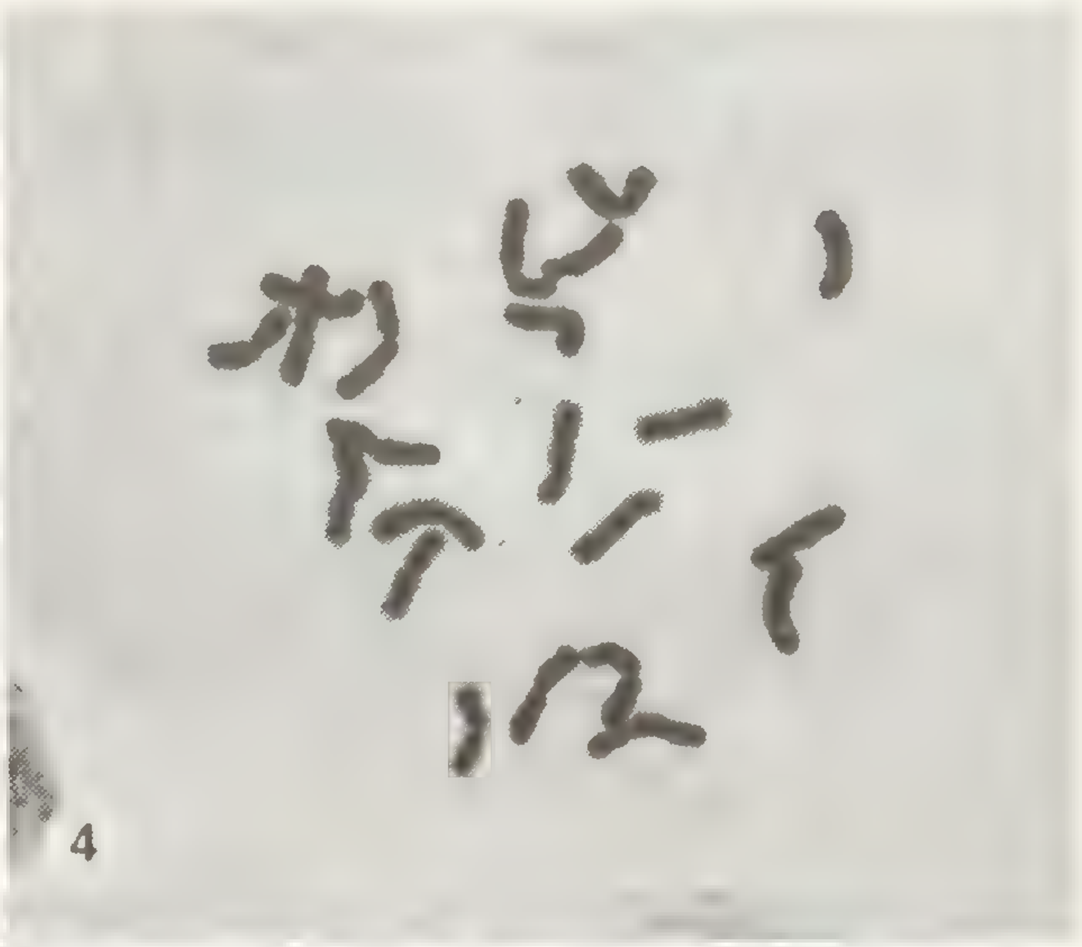
The pteridophytes of Hawaii have received relatively little attention from botanists during the past half-century. However, intensive recent studies in the islands have revealed a number of previously undescribed species, including the quillwort named here. Quillworts have been found in Hawaii several times since early this century. Between 25 September and 17 October 1917, Charles N. Forbes discovered *Isoëtes* from pools atop Mt. Eke on the island of Maui *(Forbes 682. M.* BISH). Forbes did not recognize his collection to be *Isoëtes.* It was not until 17 October 1977, when F. R. Warshauer collected *Isoëtes* from the east slope of Mauna Kea on the island of Hawaii *(Warshauer & McEldowney 1500* BISH), that Hawaiian botanists realized there was a native quillwort in the flora. Robert W. Hobdy again discovered *Isoëtes* in pools on the summit of Mt. Eke on 23 October 1980 *(Hobdy 931* BISH), and he collected it once more from the same site on 21 October 1982 *(Hobdy 1705-1710* BISH). Study of these specimens and three later collections from Mt. Eke *(Taylor 5332, 5658* MIL and *Palmer 672* BISH) indicates that these Hawaiian quillwort acquisitions represent a previously undescribed species of *Isoëtes*.

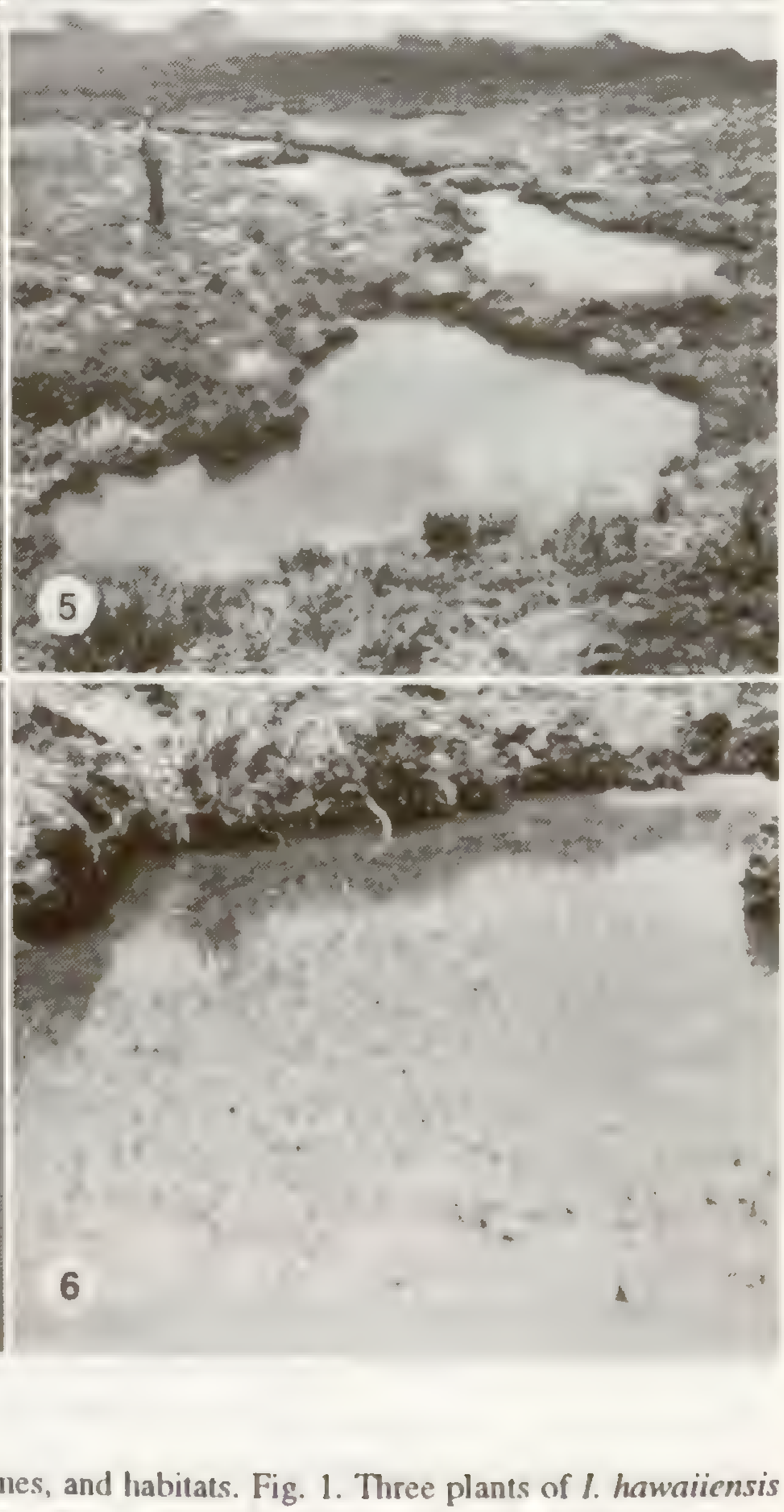
Isoëtes hawaiiensis W. C. Taylor & W. H. Wagner, sp. nov. – HOLOTYPE: Maui, pools, Mt. Eke, *Forbes 682. M.*(BISH).

Planta aquatica, emergens. Caudex brunneus, subglobosus, cylindricus, vel fusiformis, bilobatus. Radices amplae, implexae. Folia vivide viridia, basin versus pallida, semper-virentia, gracilia, usque ad 25 cm longa, ± dimidio tegens. Sporangium subglobosum ±2–3 mm diametro, pariete non pigmentifero, ±10–20 megasporas vel numerosas microsporas continens. Megasporae albae, 475–650 μm diametro, obscure vel manifeste rugulosae vel tuberculatae. Microsporae pallide brunneae, 30–40 μm longae, spinulosae. $2n$=22 (Figurae 1–4).

Emergent aquatic. Rootstock brown, subglobose to cylindrical or fusiform, bilobed. Roots extensive, matted. Leaves bright green, pale toward base, evergreen, slender, up to 25 cm long, ± 1 mm in diameter, abruptly dilated toward base to accommodate sporangium, gradually tapered to apex, spirally arranged, flaccid. Velum overlaying ±50% of adaxial wall of sporangium. Sporangium subgloblose, ±2–3 mm in diameter, wall unpigmented, containing ±10–20 megaspores or numerous microspores. Megaspores white, 475–650 μm in diameter, with obscurely to boldly rugulate or tuberculate texture. Microspores light brown, 30–40 μm in length, with spinulose texture. $2n$=22 (Figs. 1-4).







Figs. 1–6. *Isoëtes hawaiiensis* plants, spores, chromosomes, and habitats. Fig. 1. Three plants of *I. hawaiiensis* from pools on Mt. Eke. Fig. 2. SEM photomicrograph of an *I. hawaiiensis* megaspore showing rugulate texture. Bar scale = 240 μm. Fig. 3. SEM photomicrograph of two *I. hawaiiensis* microspores showing echinate texture. Bar scale = 24 μm. Fig. 4. Brightfield photomicrograph of *I. hawaiiensis* chromosomes in a single cell of a root tip squash. Chromosomes stained with Wittman's hematoxylin. Fig. 5. Shallow ponds on summit of Mt. Eke containing *I. hawaiiensis*. Fig. 6. *I. hawaiiensis* rooted in the soft mud bottom of a Mt. Eke pond.

Presently, *Isoëtes hawaiiensis* is known only from the top of Mt. Eke on the island of Maui and from the east slope of Mauna Kea on the island of Hawaii.

Mt. Eke, a peak on the northeast side of the West Maui Mountains, is shrouded in nearly perpetual mists at 1375 m elevation. Annual precipitation is estimated to be about 6000 mm. Mt. Eke's summit is the remnant core of a volcanic trachyte dome, roughly circular in shape and flat topped. Low growing bog vegetation covers most of the mountain top except where irregularly shaped shallow ponds occur (Fig. 5). The forty-seven ponds on the summit of Mt. Eke total about 0.4 hectares in area, harbor approximately 100,000 individuals of *I. hawaiiensis*, and appear to be the only habitat for *Isoëtes* in this region. Although the shallow ponds on Mt. Eke appear to hold water most of the time, some of the ponds occasionally are without standing water. Similar ponds at higher and lower elevations in the montane bogs of west Maui have not been observed to contain *Isoëtes*. On Mt. Eke, *I. hawaiiensis* grows in the soft mud bottoms of the ponds. Plants form loose clumps composed of 10–15 individuals with their roots intertwined and matted. From a distance, these aggregations give the impression of a sparse lawn (Fig. 6). The largest plants occur in water averaging 8–10 cm deep. The pond margins, which are most subject to periodic drying, are usually devoid of *Isoëtes*.

The known populations of *I. hawaiiensis* on the east slope of Mauna Kea are located in two adjacent stream channels within a montane rainforest at about 850 m elevation, approximately 17 km northwest of Hilo. At this location, about 50 to 100 plants grow close together in several patches attached to lava rock forming the stream courses. Annual precipitation in this area is estimated to be about 7600 mm.

Noteworthy features of *I. hawaiiensis* are its polymorphic rootstock and its extensive, matted roots. Rootstocks of *Isoëtes* are usually uniform in shape for a particular species. However, rootstocks of *I. hawaiiensis* may grow a centimeter or more either horizontally to become fusiform or vertically to become cylindrical. Occasionally, cylindrical rootstocks are dilated distally and are clavate, clavately lobed, or variously lobed. Roots of *I. hawaiiensis* are slender, numerous, and often longer than the rest of the plant. Such extensive roots are probably needed to anchor plants securely in soft mud. These roots are intertwined and tangled when plants are lifted from their substrate.

Isoëtes hawaiiensis is a basic diploid species. It appears to have evolved through the isolation and genetic divergence of plants brought from a distant continent or island. Progenitors of *I. hawaiiensis* possibly arrived on Hawaii as spores deposited from the bodies of migratory waterfowl. For example, the Mallard is known to eat *Isoëtes*. This duck occasionally reaches the Hawaiian Islands presumably from its breeding grounds in western North America (Berger, 1981, p. 227–233). The Canada Goose, which also has been observed to devour *Isoëtes*, is an irregular visitor to the Hawaiian lowland wetlands. While North American origins for *I. hawaiiensis* are plausible, they do not seem likely for at least two reasons. First, western North American species of *Isoëtes* appear to be quite different from *I. hawaiiensis*. Second, there are no records of ducks or geese frequenting Mt. Eke or the west forest summits in the Eke region.

It seems more likely a progenitor of *I. hawaiiensis* could have come from the *I. taiwanensis* complex. The *I. taiwanensis* complex is a group of high elevation, cold water, island species occurring along the western Pacific rim from New Zealand to Taiwan (Britton & Brunton, 1991). For example, like *I. hawaiiensis*, *I. taiwanensis* is a basic diploid. It also has rugulate megaspores and echinate microspores. Furthermore, *I. taiwa-*

nensis occurs in a small, shallow pond on northern Taiwan near the top of an extinct volcano at an elevation of about 1000 m (DeVol, 1972). Whereas *Isoëtes taiwanensis* is distinguishable from *I. hawaiiensis* by its lack of a velum, trilobed corm, and smaller megaspores and microspores which average less than 400 µm in diameter and less than 30 µm in length, respectively, *I. taiwanensis* is similar to *I. hawaiiensis* in chromosome number, spore textures, and habitat. An ancestor of *I. hawaiiensis* from the *I. taiwanensis* complex could have reached the Hawaiian Islands via the Pacific Golden Plover which winters from India, China, Taiwan, and the Hawaiian Islands south to Australia, New Zealand, Samoa, and Oceania. In spring this plover migrates to its breeding grounds on the tundra of northern Siberia (Johnsgard, 1981, p. 135). The Pacific Golden Plover is an abundant visitor to Hawaii and it frequents the shallow pools on Mt. Eke where *I. hawaiiensis* grows (Berger, 1981, p. 231).

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We are grateful for assistance from Jane A. Medler, Botany Department, Bishop Museum, William R. Anderson, Herbarium, University of Michigan, Daniel D. Palmer, University of Hawaii-Manoa, Jill K. Frey, University of Wisconsin-Milwaukee, and Chen-Meng Kuo, National Taiwan University.

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SHORTER NOTES

Ethnopharmacological Notes on Two Venezuelan *Asplenium* — During an ethnobotanical inventory in the upper Morón River drainage (10°17' – 10°28' N Lat. 68°10' – 68°16' W Long.), Carabobo State, Venezuela, information was gathered on the medicinal uses of plants from the area. The elevation of this region is between 1000–1200m, and the vegetation is a typical cloud forest of the Coastal Mountain Range. The local people use at least 41 native species; most of them are medicinal in nature, including two ferns belonging to the genus *Asplenium.* Since times remote, *Asplenium* species have been used as medicines; in fact, the very etymology of the name *Asplenium* comes from the Greek for spleen. Modern references mention various species of this genus with different medicinal uses (Boom, Amer. Fern J. 75:19–21, 1985; Wile May, Bot. Rev. 44:491–528, 1978; Murillo, Usos de los helechos, Univ. Naoc. Colombia, 1983).

We report here a remarkable case of two different species of *Asplenium* that are commonly used as medicines in our study area. *Asplenium obtusifolium* L., locally known as "Salvajito", is used as an analgesic to relieve backaches. A decoction using the entire plant is administered two or three times a day for five days *(Diaz & Niño 235* PORT, MO). *Asplenium serratum* L., locally known as "Canaguara", is used as a analgesic, for pains from bruises and contusions. A decoction of the entire plant is prepared and taken with milk *(Diaz 542* PORT, MO).

This is the first report of the use of these two ferns to treat back and muscle aches produced by trauma. As a final note, we mention that Tryon (Amer. Fern J. 49:10–24, 1959) reported that the Inca people sometimes use an unidentified species of *Asplenium* called "espinicu" as a substitute for "coca". Tryon's information, along with the new uses reported here, suggests that some type of alkaloid may be present in these and other species of the genus. — FRANCISCO ORTEGA and WILMER DIAZ, Herbario Universitario, UNELLEZ-Guanare, Programa de Recursos Naturales Renovables, Guanare 3310, Edo. Portuguesa, Venezuela.

Vasculature of the Rhizome in the Fern Genus *Dipteris* — During the course of a comparative study of the vascular organization in the homosporous ferns, some interesting morphological peculiarities in the vasculature of the rhizome in three species of *Dipteris* viz., *D. conjugata* Reinw., *D. lobbiana* (Hook.) Moore and *D. wallichii* (R. Br.) Moore were noticed. The results were presented in a National Symposium at Palayamkottai, Tamil Nadu (India) and published by Chandra S. (Indian Fern J. 9:133–137, 1992)

Recently, one of us (S.K.) had an opportunity to visit the US National Herbarium, Smithsonian Institution, Washington, DC, and through the courtesy of David B. Lellinger, procured some rhizome material of *D. chinensis* Christ (Y. Taiang 4706; US 1595383), *D. nieuwenhuisii* Christ *(P. W. Richards 2133;* US 2139135) and *D. novoguineensis* Posth., *(L.J. Brass 29665;* US 235728). Thus, details of six species out of a total of eight species of the genus are available, the remaining two being *D. papilioniformis* Kjellberg from Celebes and *D. quinquefurcata* (Bak.) Christ from Borneo which could not be studied due to a lack of material.

The observations made on the three species procured from US National Herbarium are presented here, and the present investigation also aims to explain the possible relationship

among the six species studied and to assess how far these attributes are of potential value in taxonomic and phylogenetic considerations. The rhizome in all three species is slender, wide-creeping and dichotomously branched like the three species studied earlier. The fronds are monomorphic and borne in two rows on the dorsal surface of the rhizome. The vascular cylinder of the rhizome is solenostelic with large pith and is similar to the three species of *Dipteris* studied earlier. The vascular supply to the leaf in all three species *(D. chinensis, D. neieuwenuisii* and *D. novoguineensis)* resembles that of *D. conjugata,* where each leaf trace consists of a solitary, C-shaped, ribbon-like strand that is undivided up to a point close below the base. The branch trace does not have any correlation with the leaf trace, and the branching of the vascular cylinder is dichotomous. The root traces originate as superficial vascular strands from any part of the outer surface of the vascular cylinder as in other three species studied earlier. Thus *D. chinensis, D. conjugata, D. nieuwenhuisii* and *D. novoguineensis* form one group of species where each leaf trace consists of a solitary, undivided, C-shaped strand indicating the relatively primitive condition of the vasculature of its axis as in *Matonia, Gleichenia* and *Cheiropleuria.*

D. lobbiana shows a sign of advancement where the leaf trace arises as two separate strands, one from either margin of the leaf gap, which further divide into four vascular strands before entering the leaf base. Further increase in the number of vascular strands constituting a leaf trace has been reported in *D. wallichii* where the leaf trace originates as four separate strands, two from either margin of the gap, which may later divide to form eight vascular strands before the trace leaves the cortex of the axis. On the basis of the vasculature of the rhizome, it is suggested that the complexity of the leaf trace in these two species may be held as forming a series progressive from the simple condition *(D. chinensis, D. conjugata, D. nieuwenhuisii* and *D. novoguineensis)* through *D. lobbiana,* leading to the more complex state in *D. wallichii* which probably exhibits a derived condition. Panigrahi & Patnaik (Amer. Fern J. 53:42–46, 1963) reported *D. wallichii* as tetraploid (2n=66) which further strengthens the evolutionarily advanced condition of the species — SUBHASH CHANDRA AND SURJIT KAUR, National Botanical Research Institute, Lucknow-226001 (India).

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Germination of Stored Spores of *Pteris vittata* L.

AMBIKA BERI and S. S. BIR
Department of Botany, Punjabi University, Patiala – 147002, India

Fern spores offer an excellent biological system for the analysis of physiological and developmental criteria. The independent gametophytes of ferns and their morphological simplicity have enabled us to use them as experimental objects in certain areas of plant growth and development. However, viability of spores of different ferns varies enormously with periods ranging from a few days to a few years (cf. Miller, 1968). Spores deteriorate during storage as revealed by reduction in their germination percentage (Conway, 1949; Stone, 1961; Klekowski, 1970; Bir & Goyal, 1981) and in the pace of gametophyte development after germination (Towill & Ikuma, 1975). The changes during spore germination involve mostly the breakdown of storage reserves present in the spores. The studies on the loss in viability of spores during storage are not well documented. The aim of the present investigation is to determine the physiological and biochemical changes that take place in spores during storage.

Results on spore germination, protonemal development and biochemical changes during germination of spores of *Pteris vittata* L., a fern common on the Indian subcontinent, are presented here.

MATERIALS AND METHODS

Fertile fronds of *Pteris vittata* L. (tetraploid sexual plants with n=58) were collected from the green house of Botanic Gardens, Punjabi University, Patiala. The fronds were cut into pieces and placed on white smooth paper for 24 hours with fertile surface down. The liberated spores were passed through lens paper to remove the sporangial wall materials and clean spores were obtained. These were stored at room temperature during January – April, 1992 with the storage period varying between 10 days to 100 days. Average range of outside minimum and maximum temperatures at University campus Patiala being 4.6–18.6°C; 4.9–20.1°C; 15.7–27.3°C; 16.7–30.4°C for January, February, March and April respectively. Average room temperatures for these four months being 15°±2, 16°±2, 21°±2 & 24°±2 centigrade respectively. The basal culture medium of Miller & Miller (1961) as modified by Raghavan (1965) was used. For germination experiments, spores were surface sterilized with 0.1% mercuric chloride solution and washed subsequently with sterile double distilled water. Sterilization of medium was done in an autoclave at 15 lbs/inch^2 pressure for 30 minutes. All the glassware was sterilized with ethanol and then placed in an oven at 180–190° for 2 hours. The spores were sown in small petri dishes of 10 centimeters diameter each containing 10 ml of basal medium. Spores were sown on the surface of the medium at density of 15,000 ± 500 spores per petri dish. Basal medium was added twice at a regular interval of 5 days. The cultures were raised under continuous illumination provided by two fluorescent tubes (20 watts each) and two incandescent lamps (60 watts each) placed at a constant distance of 60 centimeters above the petri dishes and at a constant temperature of 22±1°C. Three replicates of each sample were taken. Observations on the percentage of spore germination and gametophytic growth were recorded on every 5th day up to 15 days. Spore size was measured under a microscope with an ocular micrometer at magnification of 400.

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The major and minor axis of the spore were measured and spore size was calculated from the formula proposed by Bain & Robertson (1951).

For protein estimation, alkaline hydrolysis was done by digesting 50 mg spores in 1N NaOH at 100°C for 10 minutes. The contents were cooled and centrifuged and the supernatant was used for the protein determination (Hewitt, 1958). The color was developed by method given by Lowry, et al., (1951). For the estimation of total soluble sugars and free amino acids the extraction was made in 70% ethanol and we followed Clegg (1956) and Lee & Takahashi (1966), respectively.

Results

Spore volume. – To determine the effect of storage on swelling ability of spores after incubating on the germination medium, the volume of stored spores was measured at intervals and compared with that of fresh spores. With incubation on germination medium for 24 hours and 48 hour periods, there was an increase in spore volume by 1.14 and 2.19 times, respectively, over the original size of fresh spores. A perusal of data in Table 1 indicates that during incubation the increase in volume of stored spores was significantly much lower as compared to the fresh spores.

Analyzing the volume increase on incubation for fresh and 100 days stored spores, it becomes clear that in case of fresh spore an increase in volume from 24 hours to 48 hours incubation is 119.48 percent, while in 100 days stored spore the respective increase is only 7.46 percent. Evidently there has not only been a decrease in the volume of spore (or shrinkage on drying) over long storage period, but also there is a relative decrease in its imbibition and swelling capacity on germinating medium.

Germination and Protonema Growth. – Figure 1 shows the percentage of germination of *Pteris vittata* spores after storage at different time intervals. The percent germination was determined at day 5, 10 and 15. The germination percent not only decreased gradually with increases in time of storage but was also considerably delayed. In spores stored for 100 days, germination was 12 percent, 50 percent and 60 percent at 5th, 10th and 15th days, respectively. The length of rhizoid and protonema decreased with increased storage period (Table 2). A sharp decrease in length of rhizoid and protonema of 10–15 days stored spores as compared with fresh spores was noticed. The percentage decrease in rhi-

Table 1. Spore volume variations in fresh[a] and stored spores of *Pteris vittata* L.

Period of storage	Volume of spores (μm^3) on Basal Medium	
	After 24 hours	48 hours
Fresh	57,020±612.23	109,648±2152.00
20 days	*49,633±374.64	*50,668±621.13
40 days	*42,709±534.64	*47,359±664.11
60 days	*35,380±405.45	*43,340±602.39
80	*32,460±389.52	*36,360±424.64
100 days	*32,322±324.74	*34,733±463.32

[a] Initial volume of fresh spore 49,957±310.64 μm^3.

* Difference significant

Table 2. Germination characteristics of stored spores of *Pteris vittata* L.

Number of spore storage days	Rhizoidal Length (µm)		Average number of Rhizoid		Protonemal length (µm)		Protonema cell number	
	a	b	a	b	a	b	a	b
Fresh spore	526.58 ±17.43	585.05 ±12.40	1.38 ±0.09	1.45 ±0.10	197.24 ±8.24	267.09 ±9.34	1.5 ±0.10	4.00 ±0.26
20 days	*454.90 ±18.70	575.35 ±9.71	*1.11 ±0.07	1.25 ±0.08	*159.50 ±5.87	374.82 ±18.30	*1.0 ±0.00	*2.88 ±0.16
40 days	*392.14 ±18.97	*508.34 ±16.62	*1.05 ±0.06	1.38 ±0.09	*137.41 ±8.31	274.39 ±11.34	*1.1 ±0.07	*2.61 ±0.18
60 days	*386.36 ±18.19	*496.17 ±9.52	1.21 ±0.09	1.30 ±0.09	*126.24 ±9.16	250.41 ±8.86	*1.0 ±0.00	*2.41 ±0.10
80 days	*378.48 ±11.64	*472.03 ±8.96	1.23 ±0.10	*1.20 ±0.06	*123.34 ±9.50	*243.34 ±7.35	*1.0 ±0.00	*2.03 ±0.16
100 days	*3909.11 ±12.52	*411.76 ±11.64	*1.00 ±0.00	*1.20 ±0.05	*99.00 ±8.82	*223.63 ±9.678	*1.0 ±0.00	*2.00 ±0.18

a: after 10 days of germination
b: after 15 days of germination.
* Difference significant.

zoidal length of spores stored for 100 days was 41 and 29 over fresh spores after 10 and 15 days on the nutrient medium, respectively. The protonemal length of 100 days stored spores decreased 49 and 16 percent at 10th and 15th day, respectively, as compared with fresh spores.

Average number of rhizoids and protonema cell number were also calculated (Table 2). Irrespective of the storage time, a marked difference was observed in number of rhizoids and protonema cells in fresh and stored spores. However, for different storage intervals there was not much change in the number of rhizoid and protonema cells.

Biochemical changes. – Figure 2 shows the content of total soluble sugars in fresh and stored spores. Compared with fresh spores the level of sugars decreased with storage. A gradual decrease in sugar content was observed with increase in the number of days of storage. Similar decreases were observed with total free amino acids content (Fig. 3).

The protein content of stored spores decreased gradually with increasing time of storage. A sharp decrease was observed up to 30 days of storage after which protein content declined gradually up to 80 days of storage. A marked decrease was observed at 90th and 100th day of storage (Fig. 4).

Discussion

Our results on viability of spores of *Pteris vittata* during storage indicate that there is definite decrease and delay in germination due to storage. The work of Smith & Robinson (1975) on spores of *Polypodium vulgare* showed that increasing age of the spores delays the first morphological signs of germination. Our results also coincide with the earlier report by Bir & Goyal (1981), who showed that only 55–58 percent of 12-week stored *Pteris* spores germinated. Hendricks & Taylorson (1979) stated that the loss of viability of seeds stored at room temperature may be attributed to rapid changes in the water content. In the light of this observation we can say that in stored fern spores (at room temperature) also this might be the reason for decrease in their germination.

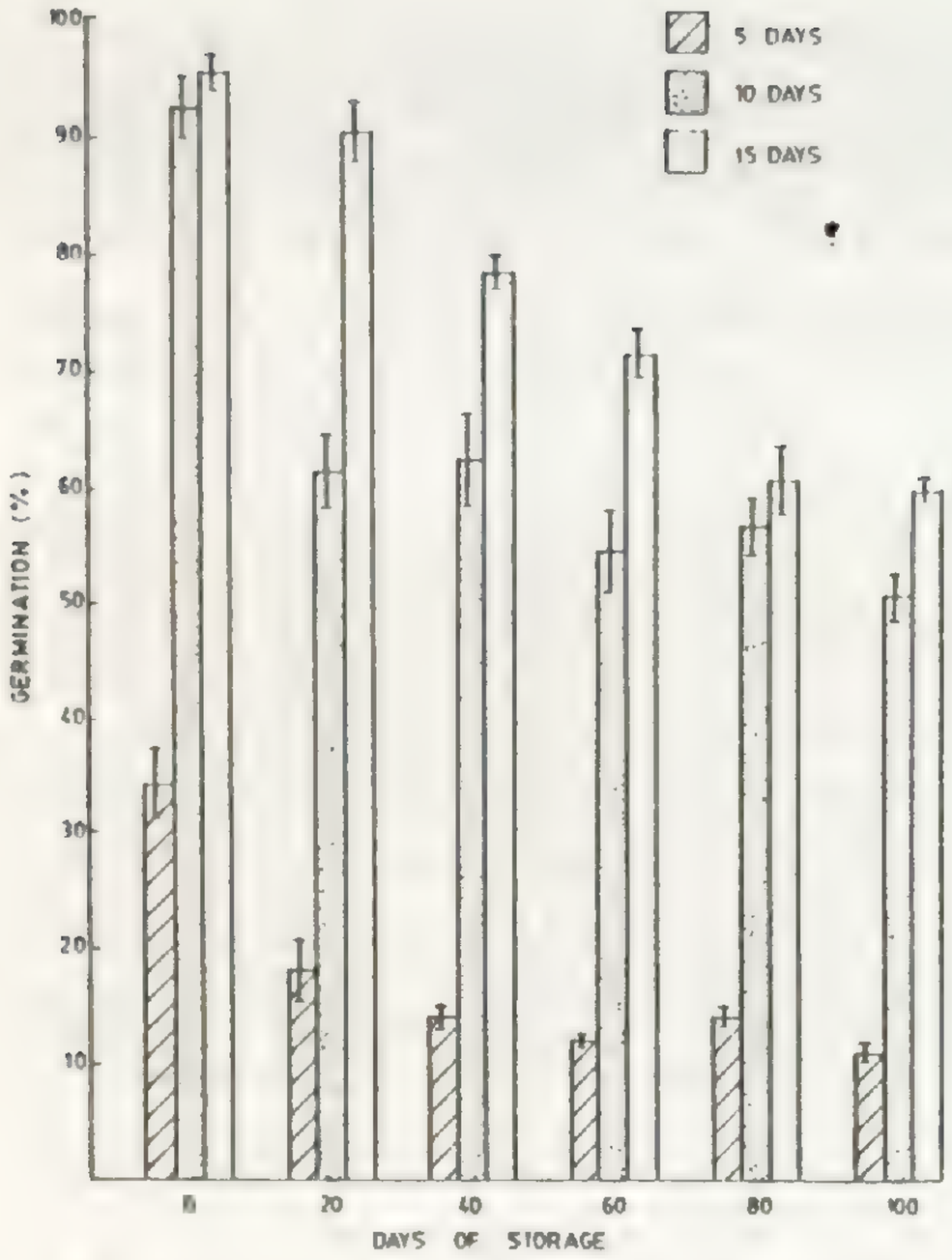

Fig. 1. Germination responses of *Pteris vittata* L. spores during storage.

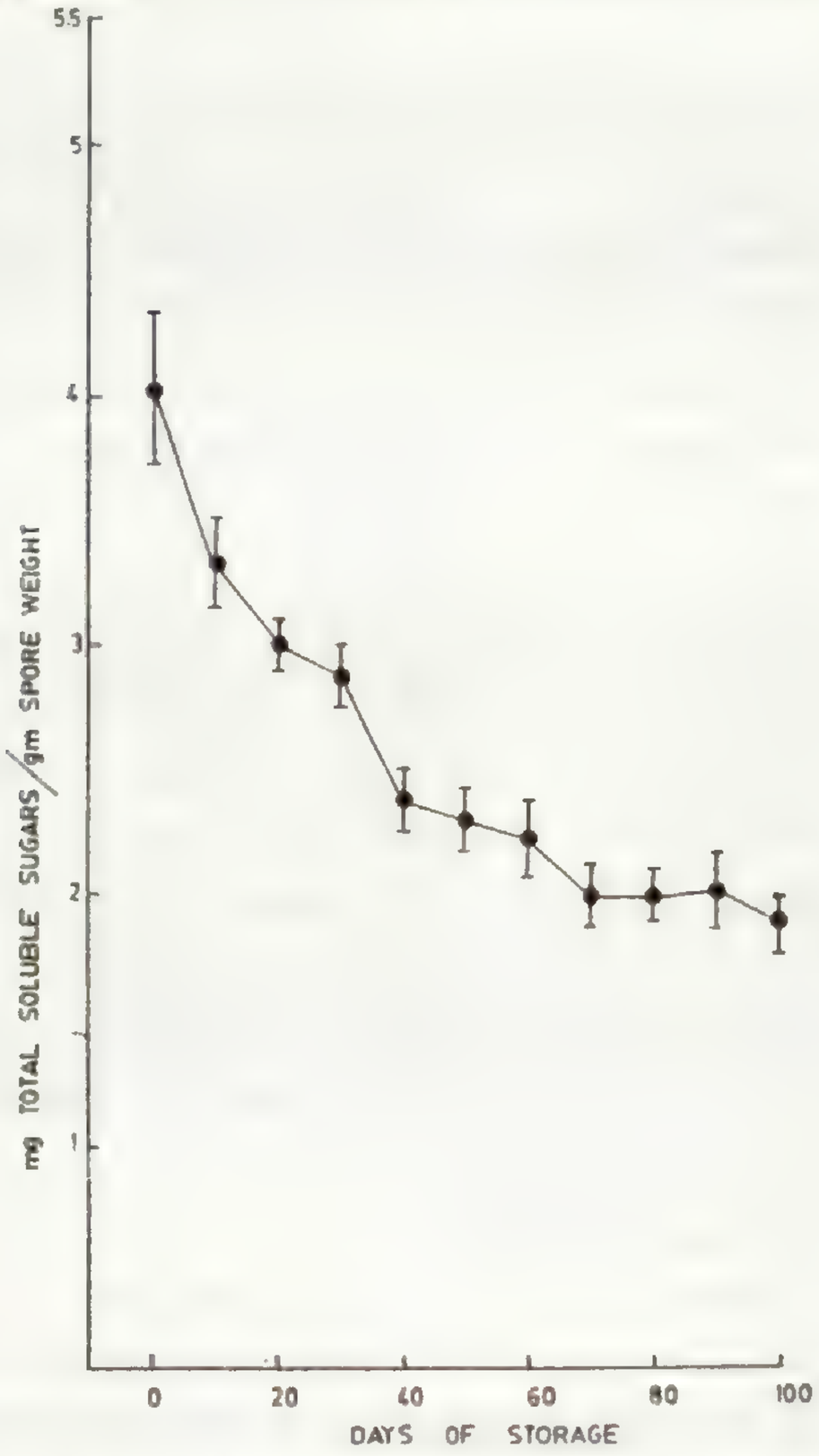

Fig. 2. Effect of storage on total soluble sugar contents of *Pteris vittata* L. spores.

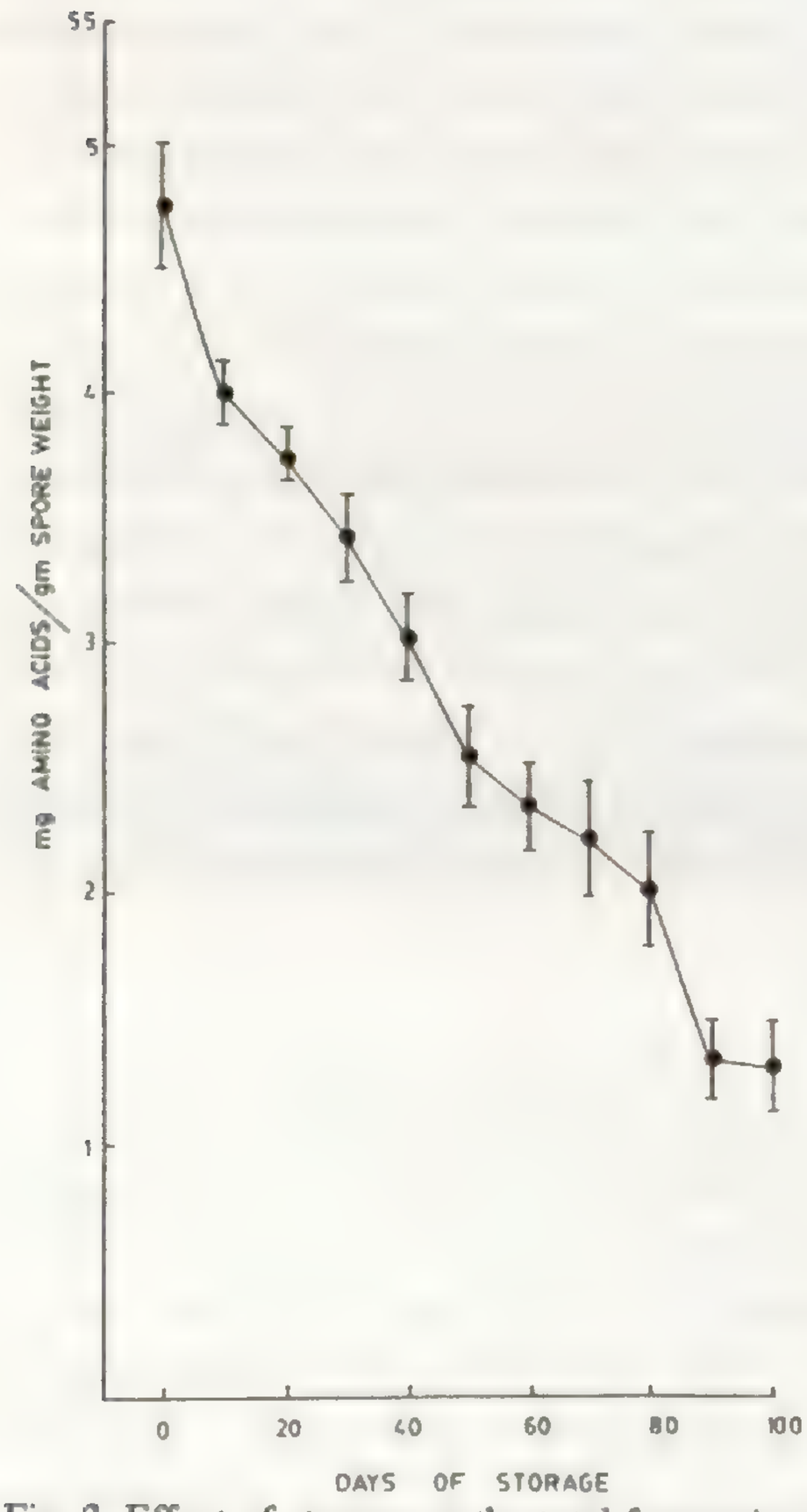

Fig. 3. Effect of storage on the total free amino acid contents of *Pteris vittata* L. spores.

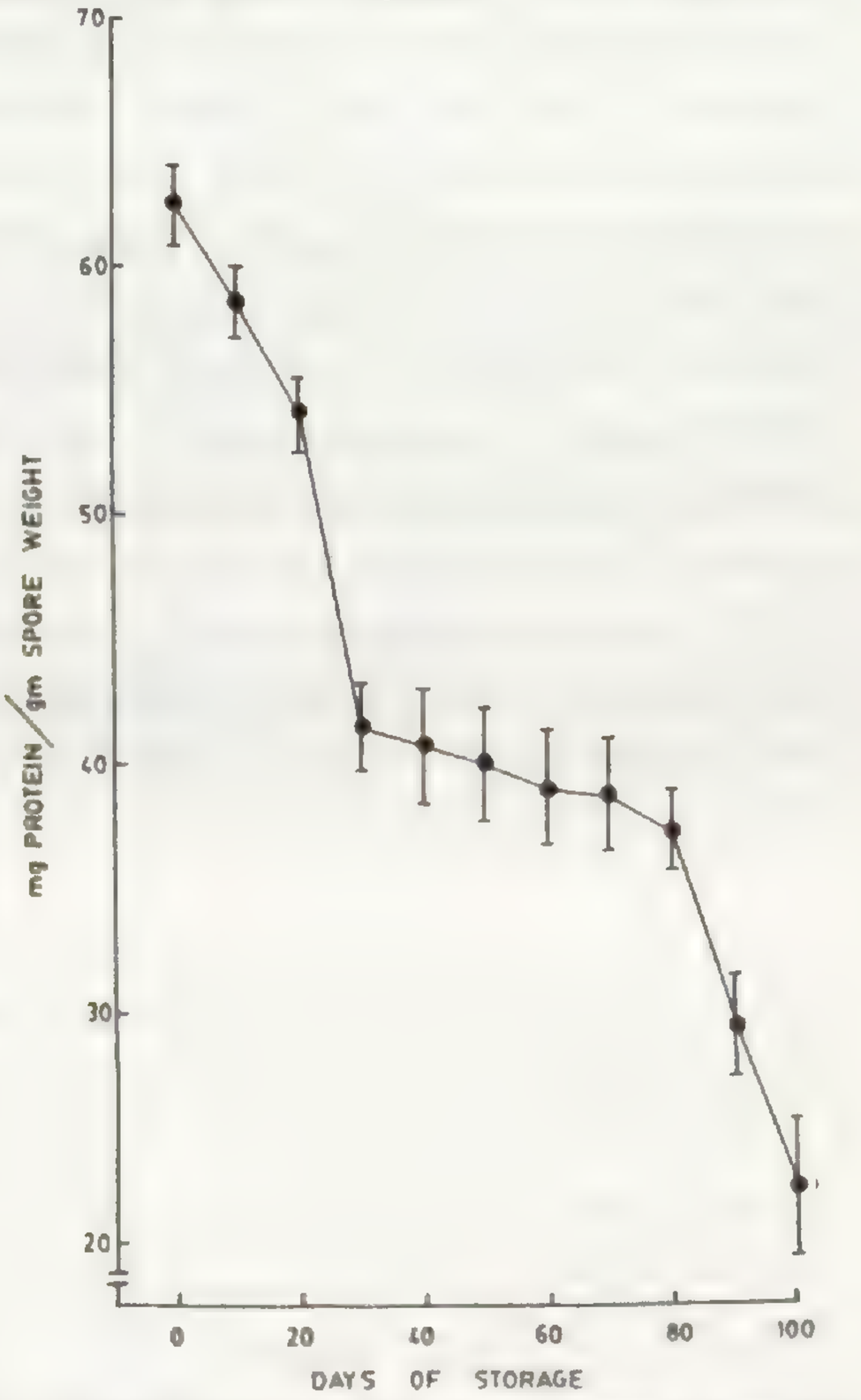

Fig. 4. Effect of storage on the total protein contents of *Pteris vittata* L. spores.

From the observations on the swelling ability of spores, it is apparent that stored spores failed to attain the size comparable with that of fresh spores resulting in their reduced viability. Grewal (1988) reported that increased volume during incubation in the germinating medium seems to be correlated directly with pollen viability. The ability of stored pollen samples to develop internal pressure may be reduced as a consequence of the deterioration of membrane effectiveness and lowered capability for maintaining required turgor (Heslop-Harrison, 1979).

The inhibition of protonemal elongation and rhizoidal length of spores stored for different time intervals may be related to the metabolic activity of stored spores. Metabolic activity of spores continued even though at a very reduced rate after dispersal, thus it is expected that endogenous substrates needed for germination and further growth would be gradually used up as in case of pollen (Wilson, et al., 1979).

Biochemical analysis of fresh and stored spores indicated loss in the content of soluble sugars, total free amino acids and proteins (cf. Figure 2–4). Various biochemical studies on seeds (Bewley & Black, 1982) have also shown that the loss of viability is associated with many biochemical impairments. Earlier reports on pollen indicate that the major cause of loss of viability with storage may be due to deficiency of respiratory substrates and irreversible loss of membrane permeability (Shivanna & Heslop-Harrison, 1981; Grewal, 1988) as well as inactivation of various enzymes and growth substances (Mcleod, 1975; Chhabra & Malik, 1978; Setia & Malik, 1984). We may thus infer that due to homology between pollen and spore systems, the foregoing factors could be the cause of loss of viability of fern spores also during storage.

Summing up we may state that the present studies demonstrate that reduced spore viability during storage causes decrease in germination percentage and protonema elongation because of decrease in reserve substances viz-a-viz total soluble sugars, free amino acids and protein contents.

Summary

The effects of storage at room temperature on spores of *Pteris vittata* L. are studied. In case of stored spores, germination percentage and protonemal tube length decline markedly. There is also marked decrease in number of rhizoidal and protonemal cells of stored spores when compared with fresh spores. Stored spores when put in germination medium fail to attain the size comparable to fresh spores. Biochemical analysis of fresh and stored spores indicated gradual loss in the contents of total soluble sugars, total free amino acids and proteins on storage. Thus reduced germination of stored fern spores appears associated with biochemical impairments.

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Spore Germination and Early Gametophyte Development of *Platycerium bifurcatum*

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Fern spores and gametophytes are excellent biological systems for the analysis of physiological and developmental problems (Raghavan, 1989). Gametophytes provide opportunities for studying various aspects of growth and differentiation (Hickok et. al., 1987). Furthermore, their existence is a boon to fern genetics (Chasan, 1992). An *in vitro* culture technique has been used to study different aspects of germination and early gametophyte development in many fern species (Nester & Coolbaugh, 1986; Von Aderkas & Raghavan, 1985; Whittier, 1981). Considerable information has also accumulated on the effect of different substances on spore germination and gametophyte growth (Melan & Whittier, 1990; Miller & Wagner 1987; Miller et al., 1983).

In *Platycerium,* these kinds of investigations have received little attention. Either the pattern of initial cell divisions during germination of spores was investigated (Nagmani & Raghavan, 1983) or older developmental stages of gametophytes were studied (Thentz & Moncousin, 1984; Camloh & Gogala, 1992). Therefore, in this investigation the process of spore germination and early gametophyte development of *Platycerium bifurcatum* was followed in detail. The effects of different culture conditions on various developmental stages of germination were examined. Stained whole-mount preparations were used for a more exact determination of cell divisions.

MATERIAL AND METHODS

Spores of *Platycerium bifurcatum* (Cav.) C. Chr. were kindly provided by Dr. B.J. Hoshizaki, University of California. They were harvested in her garden in Los Angeles in September 1991 and stored at 5°C in the dark until use. Spores were isolated from sporangial debris by filtering water suspensions of impure spores through a 60 μm nylon filter (Nybolt PA-60/33). They were collected on an 11μm nylon filter (Nybolt PA-11/4/C) and sterilized, unless indicated otherwise, first by a 5s exposure to 70% ethanol, followed by a 10 min. exposure to 10% (v/v) commercial bleach (4% NaOC1) with a drop of Tween 80 added, and thoroughly rinsed with sterile water. The water used for the sterilization procedure and media preparation was purified with a Milipore purification system (Milli Ro Plus and Milli-Q Plus unit).

Approximately 1000 spores were sown for each replicate on the surface of 3ml Miller medium (Miller & Miller, 1961) in which Fe citrate was replaced with NaFeEDTA. The pH was adjusted to 5.7-5.8 before autoclaving. The combinations of added Difco-Bacto agar and sucrose as well as the sterilization are presented in Table 1. For solidified media Petri dishes (diameter 3cm) were used. Liquid media were placed in tubes with 16mm diameter covered by aluminum caps. Cultures were maintained at 23±2°C, under a 16h photoperiod at 8.1–10.8Wm^{-2}, provided by 65-Watt fluorescent lamps. The spores were kept in these conditions from 3 to 10 days.

The germination percentage was determined 3 and 6d after spore sowing. The definition of spore germination was the emergence of a rhizoid through the spore coat. One hundred spores per replicate were scored. The length of the first rhizoid was measured 4,

Table 1. The % agar and sucrose used in different media. Spore sterilization is also indicated.

medium	A	B	C	D	E	F	G*
agar (%)	0.8	0.6	0.4	0	0	0	
sucrose (%)	3	3	3	0	3	0	0

*unsterilized spores

6 and 8d after sowing the spores and the gametophyte cell number was determined after 6, 8 and 10d of culture. For the determination of rhizoid length and number of cells, 25–35 samples were examined per replicate.

There were 2–3 replicates per treatment and all experiments were repeated twice. All parameters were scored on fresh material with a Carl Zeiss microscope. The Student t-test was used to calculate the levels of statistical significance (p) between the data obtained in different media. The mean values and 2SE are shown in tables and histograms.

For detailed observation of cell divisions, spores were sown on medium F in tubes with a 24mm diameter and plastic caps. In 1–2d intervals during the period from the second to the tenth day gametophytes were cleaned and stained with acetocarmine-chloral hydrate according to Edwards & Miller (1972). Three to four hundred gametophytes were examined each time. Photomicrographs were obtained by an Opton Axioskop.

Results

The % of spore germination 3 and 6d after sowing on different media are shown in Table 2. The germination percentages obtained after 3 and 6 days were similar. The best germination occurred when unsterilized spores were used (G) and the germination percentage was significantly higher ($p<0.05$) than in most of the other media. On the solid media (A–D), no significant differences on spore germination were observed in spite of

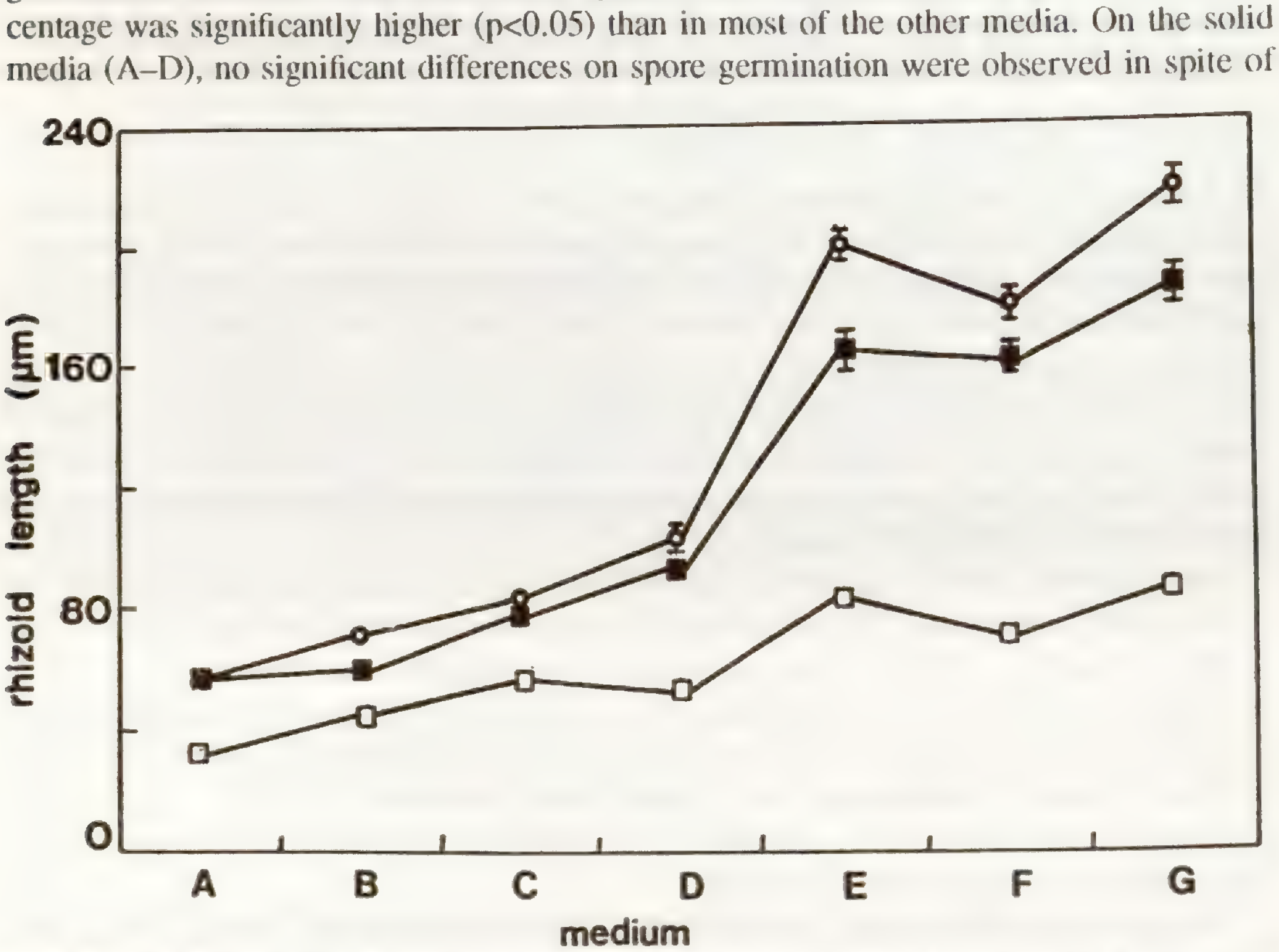


Fig. 1. Effect of various media on rhizoid length after 4 (open squares), 6 (solid squares) and 8 (open circles) days in culture (for medium labels see Table 1). Symbols without error bars had standard errors smaller than the symbols.

Table 2. The effect of various media and sterilization on spore germination. Percentage of spore germination determined 3 and 6d after sowing. Data represents mean values from 12–3 replicate samples ± SE.

medium[a]	% germination 3d	6d
A	56.0±2.2	66.0±3.8
B	54.4±0.8	73.0±2.2
C	52.4±5.3	64.1±0.3
D	53.5±5.5	67.6±6.0
E	47.3±1.6	59.8±3.8
F	50.3±3.6	68.8±1.4
G	68.2±3.6	87.2±0.6

[a]for medium labels see Table 1.

various agar and sucrose concentrations. On liquid media (E and F), the germination percentages were slightly lower (after 3d) or similar (after 6d) to the solid media.

Rhizoid elongation was greatly influenced by the medium (Fig. 1). The presence of agar in the medium greatly inhibited rhizoid elongation which can be seen not only from comparisons between agar-free and solid media but also from the increased rhizoid length at reduced agar concentrations. When 0.4% agar was added to the medium (C), the rhizoids were significantly longer ($p<0.001$) than in media with higher concentrations (A and B). The average rhizoid length of gametophytes grown for 6 or 8d in liquid media increased 2–3 fold as compared to solid media.

The absence of sucrose in the solid media (D) significantly ($p<0.001$) increased the rhizoid length, but not in liquid media. It is worthwhile to mention that the unsterilized

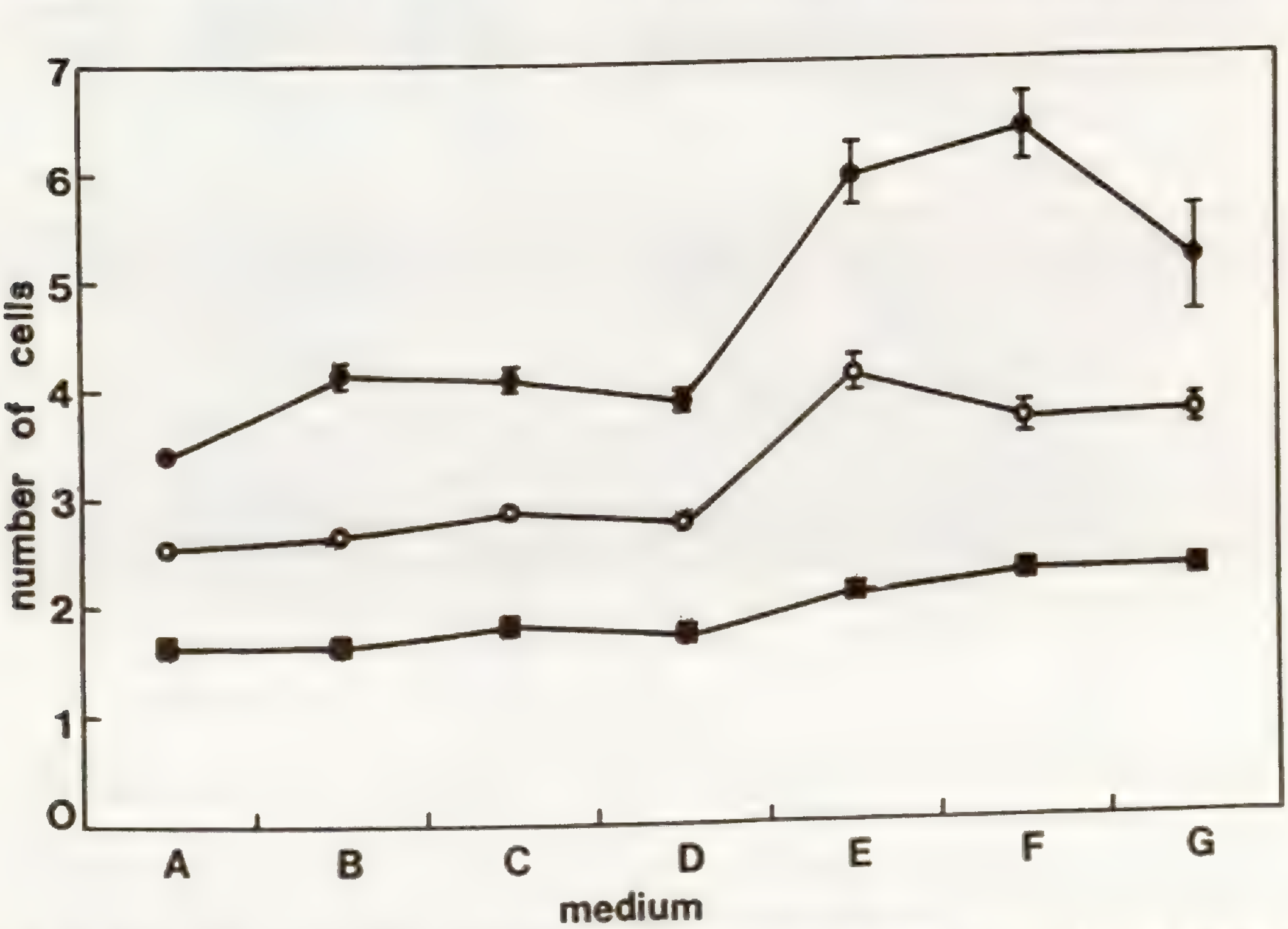


Fig. 2. Effect of various media on gametophyte cell number after 6 (solid squares), 8 (open circles) and 10 (solid circles) days in culture (for medium labels see Table 1). Symbols without error bars had standard errors smaller than the symbols.

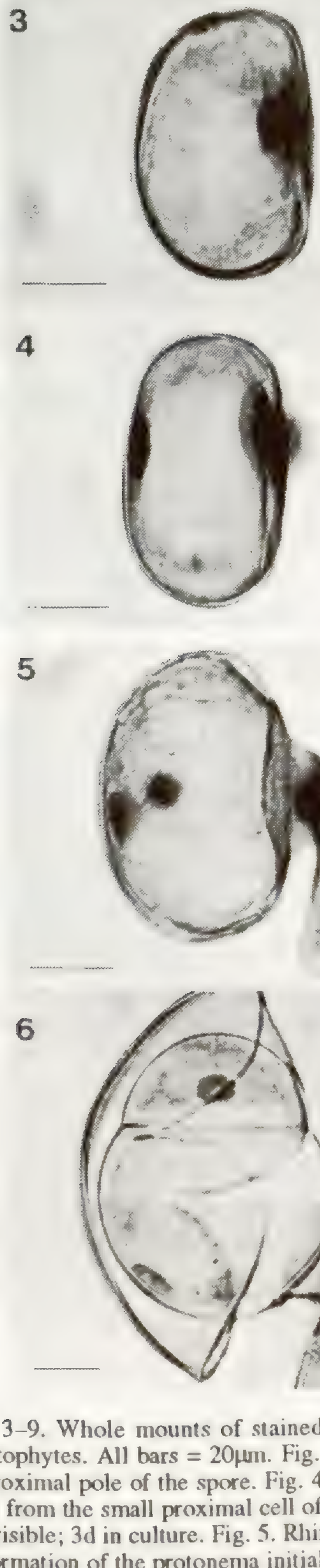
spores in liquid media had the longest rhizoids. No morphological rhizoid abnormalities were observed during these experiments.

The different media also affected the gametophyte cell number (Fig. 2). On liquid media gametophytes have a significantly ($p<0.001$ or $p<0.01$) higher number of cells than on solid media. The difference between liquid and solid media was visible after 6d in culture and increased considerably to the end of the experiment. Among the agar concentrations tested, a significantly lower number of cells ($p<0.001$) was obtained only after 10d of growth on the medium with 0.8% agar (A). The presence of sucrose had no significant effect on cell numbers on solid media (B and D), while in liquid media (E and F) the significantly higher cell numbers were obtained only after 8d in a sucrose enriched medium (E) ($p<0.05$). The cell number from unsterilized spores after 6 and 8d was comparable to sterilized spores, while after 10d it was lower.

The dry spores of *P. bifurcatum* are tan, slightly kidney-shaped with a smooth exine. After 2d in culture the nucleus was generally at the proximal pole of the spore and in some spores it had already divided (Fig. 3). After the division of the nucleus, the first asymmetric division of the spore cell occurs which yields the rhizoid initial. The rhizoid initial which grew through the exine at the proximal pole, represented the first microscopically visible sign of germination and was seen in a majority of spores after 3–4d in culture (Fig. 4). The rhizoid initial elongated and formed the primary rhizoid (Fig. 5). After approximately 6d in culture, the larger cell arising from the first asymmetric division divided asymmetrically by a wall parallel to the polar axis of the spore and gave rise to a protonemal cell (Fig. 6). This cell divided further until the protonemal filament was three to four-celled after about 8d in culture. At the same time a second rhizoid usually developed (Fig. 7). After 10d in culture, longitudinal cell divisions were commonly observed in the protonemal filament and were initiated more often in the subterminal than in the terminal cells (Fig. 8). The wall of the longitudinal division was oriented at 90° to the earlier filament walls or was displaced slightly to one side. The formation of twin protonemata accompanied with rhizoid development was also observed (Fig. 9). After 10d in culture as high as 6.2% of germinated spores generated two protonemata (data not shown).

Discussion

The experiments showed that liquid media were superior to solid media for early gametophyte development, but there was no effect on germination percentages. Thentz & Moncousin (1984) have also found that agar (3–10g/1) and sucrose (5–30g/1) did not affect spore germination of *P. bifurcatum* after 10d in culture. However, Raghavan (1989) reported that in general liquid media were superior for fern spore germination and that it was likely that solid media affect spore germination by preventing the breakage of spore coats and the outgrowth of rhizoid and protonema initials. Since in our study germination was defined as emergence of a rhizoid out of a spore coat, and we obtained even slightly lower or similar germination percentages in liquid as in solid media, it is obvious that solid media did not prevent the breakage of spore coat. However, from results obtained on rhizoid elongation and protonemata development (Fig. 1 and 2) it is apparent that solid media have an inhibitive effect on both processes. Since specific ions are important in the germination and development of fern spores (Miller & Wagner, 1987; Haupt, 1985) the reason for differences in rhizoid elongation and protonemata development obtained in



Figs. 3–9. Whole mounts of stained preparations of germinating spores and early developmental stages of gametophytes. All bars = 20μm. Fig. 3. Spore after 2d in culture. The nucleus which has already divided is at the proximal pole of the spore. Fig. 4. Appearance of the rhizoid initial outside the exine. The rhizoid is developing from the small proximal cell of the first asymmetric division of the spore. The nucleus of the distal cell is also visible; 3d in culture. Fig. 5. Rhizoid is elongating. The nucleus of the distal cell has already divided before the formation of the protonema initial; 4d in culture. Fig. 6. Germinating spore showing rhizoid and protonemal cell; 8d in culture. Fig. 7. Two rhizoids developing on a protonema with a few cells; 8d in culture. Fig. 8. Longitudinal division has already occurred and is clearly visible in the subterminal cell; 10d in culture. Fig. 9. Formation of twin protonemata accompanied by rhizoid development. Division in both cells to form filaments are visible; 10d in culture.

various media may lie in the altered diffusion of nutrients in different physical states of the medium.

From our results it is evident that sucrose has no promotive effect on either spore germination or early gametophyte growth, except for rhizoid elongation in solid media. Probably spores have enough endogenous carbohydrates for germination and early gametophyte development. This is not valid for growth of older gametophytes which gave optimal growth at 4% of sucrose in the medium (Camloh and Gogala, 1992).

The best germination was obtained when unsterilized spores were used. This might be due to the use of ethanol and NaOC1 in the sterilization process. Various alcohols have been reported to delay or inhibit germination of various fern spores (Miller, 1987), while Ca^{2+}, which is essential for spore germination, is extracted from *Onoclea* spores by NaOC1 (Miller & Wagner, 1987). We must also point out that even if the germination and rhizoid elongation were best when unsterilized spores were used, contamination always occurs especially after 10d of culture. This contamination probably was the reason for the lower cell number obtained 10d in culture as compared to the other liquid media using sterilized spores. On the other hand, agar contains many organic and inorganic contaminants (Pierik, 1987) and maybe some of them cause at least part of the differences between liquid and solid media as well as between media with different agar concentrations.

The morphology of germination obtained from stained whole-mount preparations and living spores is similar as described for *Platycerium coronarium* and *P. veitchii* (Nagmani & Raghavan, 1983) even though the spore sectioning is needed for confirmation of these observations. The exception is the occurrence of the first rhizoid initials already after 2 days in culture and that after 3 days about half of the spores develop rhizoid initials or elongating rhizoids (Table 2). Nagmani & Raghavan (1983) reported that the rhizoid initial occurs 4 to 6 days after exposure to white light for 12h but they first imbibed spores in the dark for 48h. These differences are probably due to the different species used as well as to the different light conditions including photoperiod and light intensity.

The first longitudinal divisions were observed in three to four-celled gametophytes and these divisions were initiated more often in subterminal than in terminal cells, although Raghavan (1989) reported that in general the terminal cell divides by an oblique or longitudinal wall to initiate planar morphology and that a division wall only occasionally appears in the subterminal cell. The development of twin protonemata accompanied with rhizoids was another interesting phenomenon observed during the experiment. According to Miller & Greany (1976), treatment of *Onoclea sensibilis* spores with methanol induces the formation of twin protonemata as a result of symmetrical division of spores while differentiation of rhizoids is delayed. Maybe in our experiments ethanol used for spore sterilization had some effect on the development of twin protonemata.

As discussed above, it is clear that culture conditions are important for early gametophyte growth of *P. bifurcatum*. In conclusion, spore sterilization is needed to study more than a few day old gametophytes; liquid media are superior for gametophyte growth when compared to solid, and sucrose is not required for early gametophyte growth. The optimal culture conditions as determined in this study represent an experimental system appropriate for further developmental and physiological studies of *P. bifurcatum* spore germination and gametophyte development.

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Notes on the Rediscovery, Status, and Ecology of the Very Rare Hawaiian Fern *Christella boydiae* (Thelypteridaceae)

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The genus *Christella* (Thelypteridaceae) is comprised of approximately 50 species distributed predominantly in the Old World tropics but also in the Americas (Holttum 1977). Two native *Christella* species, *C. cyatheoides* (Kaulf.) Holttum and *C. boydiae* (Eaton) Holttum occur in Hawai'i (Wagner, 1981). *Christella cyatheoides* is a common and characteristic component of matted fern understory of Hawaiian rain forest. *Christella boydiae* is a much rarer diminutive species of rocky stream courses. *Christella boydiae* has been collected only from the Ko'olau range of O'ahu, Kipahulu and Waiho'i Valleys on East Maui, and along low elevation watercourses near Hilo on Hawai'i. Currently it is only known from one of these historic populations at Kipahulu Valley.

There are 20 specimens of *C. boydiae* in the Bernice Pauahi Bishop Museum Herbarium Pacificum (BISH) that represent 18 or fewer collections. Most of these specimens are in poor condition and as typical of collections made prior to 1910, have little or no collection information. The species has been most often collected on O'ahu. Of these, six specimens (one third of the total at BISH) were made between 1900 and 1908 by either of the noted Hawaiian botanists, C.N. Forbes or J.F. Rock, mostly from the Punalu'u region of the Ko'olau range. The last collection of *Christella boydiae* on O'ahu was made prior to 1908. However, Warren H. Wagner found that this species was still present in the Punalu'u area of the Ko'olau range on O'ahu as late as 1965 (Kaluanui Stream, rock shelves, Castle trail, Hau'ula Forest Reserve, Ko'olau range, *W. H. Wagner and S. Carlquist 65270* MICH). Since that time, many exotic weeds, noteably *Clidemia hirta* (Melastomataceae), have invaded the area and this species has apparently disappeared. On Hawai'i island, the species, to our knowledge, has been collected only once. Until recently, *Christella boydiae* had been collected only three times on Maui, first in 1881 in Kipahulu Valley (*F.L. Clarke s.n.*, BISH) without further location information. Then after 86 years, *C. boydiae* was again collected in Kipahulu Valley in 1967 (*R. DeWreede 30*) along Palikea stream at 915m (Lamoureux, 1968). A third Maui collection was made in 1973 in adjacent Waiho'i Valley as part of the NSF-sponsored Waiho'i Valley Research Project (*B. Harrison 10*) at 915–1040 m along Waiohonu stream where it was a rare fern from a shady location under a ledge associated with hymenophyllaceous ferns and various mosses along the stream bank (B. Harrison-Gagne, pers. comm.). Neither of these collections is present in the cabinets at the Herbarium Pacificum (BISH): although the latter is in the University of Hawai'i collection. Fourteen specimens at the Herbarium Pacificum (BISH) include the year of collection. Of these, eight were collect-

ed before 1900 and six between 1900 and 1908. No collection of *C. boydiae* in the Herbarium Pacificum was made after 1908.

In 1985, *Christella boydiae* was rediscovered along streambeds in middle Kipahulu Valley, Haleakala National Park. Botanical specimens collected as part of baseline vegetation work were identified as *Christella boydiae* by Wagner from the University of Michigan while a visiting professor in Hawai'i in 1987. Based on subsequent field work, this species is now known from the three primary periodic watercourses of Kipahulu Valley at 1160–1500 m (*A.C. Medeiros 795, R.W. Hobdy & A.C. Medeiros 2834* through *2847*, BISH). Currently, 146 plants in six populations are known from Kipahulu Valley. Of these, 12 occur in one population on Koukouai stream, 16 in one population in a tributary of Palikea stream and 118 plants in four populations along the unnamed central valley stream. The populations of *C. boydiae* found in Haleakala National Park are the only ones known, though it may also occur outside the Park in adjacent Waiho'i Valley. The large remote tracts of stunted rain forests and stream courses in Waiho'i Valley contain substantial populations of alien plant species and semi-domestic cattle and feral pigs.

In Kipahulu Valley, *Christella boydiae* occurs on exposed, rocky, moss-covered banks of periodic stream courses in dense *Metrosideros* or *Metrosideros* and *Acacia* rain forest with a thick matted fern understory. *Christella boydiae* is a small rosette fern that grows in rock crevices and along rocky ledges mostly at, or just above, high water level. The species grows in near full sunlight and seems to avoid habitats where there is competition for space from taller species. Most plants are less than 0.25 m in height and with their small stature, any amount of such competition would result in overtopping. In winter storms, these stream courses are subject to flooding that scour the banks with high water, logs and debris. Annual rainfall in this area is approximately 250 inches (6250 mm). Although dense mist and clouds are frequent, extended periods of sunny clear weather also occur, primarily in summer. Associated native species include the ferns *Athyrium microphyllum, Amauropelta globulifera, Asplenium unilaterale, Christella cyatheoides,* and *Sadleria pallida,* the graminoids and forbs *Carex alligata, Deschampsia nubigena,* and *Pilea peploides* and the dwarfed woody species, *Broussaisia arguta, Clermontia arborescens, Metrosideros polymorpha,* and *Vaccinium calycinum.* Alien species on the stream banks include *Deparia petersenii, Holcus lanatus, Hypochoeris radicata,* and *Prunella vulgaris.*

According to the scanty record afforded by herbarium specimens, *Christella boydiae* was formerly found along streams down to very low elevations but by the 1920s had disappeared from much of its range and entirely from lower elevation habitats. The type collection of this species was made on O'ahu at 30 m elevation (Eaton, 1879). Robinson (1913) cited the habitat of his species as "on rocky ledges, along streams." Hillebrand (1888) states, "On bare rocks in the bed of Wailuku river, Hilo, Hawaii, where the ordinary sized form grows along the banks . . . Found also by Baldwin under the same conditions by the side of a stream in Koolau, Oahu." *Christella boydiae* was described from O'ahu as *Aspidium boydiae* Eaton (Eaton, 1879). Hillebrand (1888) considered it a dwarfed form of the more common native species *Christella cyatheoides* (known then as *Aspidium cyatheoides),* reduced it to a variety of that taxon (var. *depauperatum* Hillebr.), and reported the distribution as O'ahu and Hawai'i. Robinson (1913) restored this taxon to species level but in a separate genus, *Cyrtomium boydiae* (Eaton) Robinson, based on the presence of peltate indusia. Iwatsuki (1964) assigned the species to the genus

Table 1. Morphological comparison of *Christella boydiae* and *C. cyatheoides*, based on specimens of the two species collected from Kipahulu Valley, East Maui.

Character	*C. boydiae*	*C. cyatheoides*
1. Sori	medial on vein impressed below, embossed above	basal on vein surface smooth above and below
2. Indusia	peltate, 1.0–1.5 mm diam.	reniform, 1.0 mm diam.
3. Scales	absent on leaf axes	abundant on rachis and costa
4. Glandular hairs	absent	moderate to abundant below; yellowish
5. No. of layers of anastomosing veins	usually 1, sometimes 2	usually 4–6, sometimes 3–8
6. Fertile frond length	6–32 cm	20–200+ cm
7. Pinna dimensions	13–40 mm long, 5–8 mm wide	70–210 mm long, 15–28 mm wide
8. Pinna margins	crenate	strongly crenate to lobed, 1/2 way to costa
9. Pinna apices	rounded to acute	acute to acuminate
10. Pinna bases	cuneate	truncate to cordate;
	on prominent 1–3 mm stalks	on inconspicuous 1–2 mm stalks

Thelypteris as the sole representative of the subgenus *Cyrtomiopsis*. Holltum (1976) placed the species in *Christella*, a segregate of *Thelypteris*. Other authorities place the species in the large more inclusive genus, *Thelypteris*.

Due to the rarity of this taxon in the field and the consequent sparsity of herbarium collections, even recent workers have been cautious in recognizing *Christella boydiae* as distinct from the other Hawaiian species, *C. cyatheoides*. Holttum (1977) states, "Hillebrand considered this to be a depauperate form of *Christella cyatheoides*; I agree that they are allied, but think it possible that *C. boydiae* is distinct, as its scales appear to differ. Plants should be cultivated, to see what size they will attain and for a careful comparison with living plants of *C. cyatheoides*." The two Hawaiian *Christella* species are easily distinguished and when more closely examined are marked by a number of characters that clearly separate them (Table 1). In addition, *Christella cyatheoides* is a much larger fern that grows in the dense matted fern understory of Hawaiian rain forests. It is also commonly pendant along the sides of watercourses. Dwarfed individuals of *C. cyatheoides* also occur in rocky areas of stream courses. However, despite the suggestion of earlier taxonomic literature, even stunted individuals of *C. cyatheoides* are easily distinguished from *C. boydiae* (Table 1). Both species occur side by side in Kipahulu with no signs of F_1 hybridization or introgression.

The disappearance of *C. boydiae* may be due to the dense growth of alien plant species that now occupies many lower elevation stream courses. Species such as *Ageratina adenophora, Deparia petersenii, Brachiaria mutica,* and *Melinis minutiflora* are now dominant species in low elevation habitats where formerly *C. boydiae* occurred. The invasion of riparian habitats by alien weeds in the relatively high elevation stream courses of Kipahulu Valley that are the last known refuge for *C. boydiae* may be the primary threat that may affect its long-term survival. Stream courses with their high light intensi-

ties and frequent disturbance are prone to invasion by alien species, even in otherwise relatively intact Hawaiian natural areas.

Despite its rarity, *Christella boydiae* has a Hawaiian name, *kupukupu-makali'i,* literally the "small-eyed *kupukupu* fern" (Pukui & Elbert, 1986). The name *kupukupu* is usually applied to the fern *Nephrolepis,* the word *makali'i* meaning "small-eyed" presumably in reference to the conspicuous, round sori of *C. boydiae.* Early Hawaiians named the other native *Christella* species, *C. cyatheoides, kikawaio;* young fronds and roots were used for food and medicine (Pukui & Elbert, 1986).

In comparison to many other rare species in Hawaiian ecosystems, the possibility for long term conservation of this species appears good. At least there is active management ongoing to perpetuate the survival of native species and ecosystems. The known populations of *Christella boydiae* and the alien weeds that are present are being monitored.

ACKNOWLEDGEMENTS

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Parahemionitis, a New Genus of Pteridaceae

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Beddome (1883) redescribed and illustrated two species of *Hemionitis* L. from India, *H. arifolia* (N.L. Burm.) T. Moore and *H. griffithii* (T. Moore) J. D. Hook & Thomson. The latter species is now known as *Stegnogramma griffithii* (T. Moore) K. Iwats. and is a member of the Thelypteridaceae. The generic affinity of the former species has remained problematical. Tryon et al. (1990, p. 245) said, "*Hemionitis arifolia* (Burm.) Moore is similar to *Paraceterach* in some technical characters, but differs in its dimorphic leaves and simple, cordate to hastate lamina with sparse indument. It is not readily included in any recognized genus." Tryon & Tryon (1982, p. 280) said that "it may be related to *Syngramma* or a paleotropical element of *Doryopteris*." Tryon & Lugardon (1991, p. 173) said that it "is an apogamous tetraploid that is excluded (from *Hemionitis*) on the basis of its distinctive morphology as well as its flavonoid composition."

According to D. B. Lellinger (in litt.), the lamina indument of *H. arifolia* is unlike that of *Syngramma* and *Doryopteris*; it appears to be more like that of *Hemionitis* and its relatives. The stipe base and rhizome indument of *H. arifolia* is also unlike that of *Syngramma* and *Doryopteris*; it appears to resemble that of *Cheilanthes* and its relatives. The venation of *H. arifolia* is unlike that of *Cheilanthes* and *Syngramma*; it resembles, but does not exactly match, that of *Doryopteris* in forming polygonal areolae without included veinlets.

Because *H. arifolia* does not fit comfortably in any existing genus of the Pteridaceae, I have thought it best to provide it with a new generic name. I previously attempted to do so (Panigrahi, 1991, 12–13), but failed to provide a Latin description, which I thought at the time was not necessary under Art. 42.1 of the Code. This is remedied below:

Parahemionitis Panigrahi, gen. nov. (Fig. 1) — TYPE (and sole species): *Asplenium arifolium* N. L. Burm. [= *Parahemionitis arifolia* (N. L. Burm.) Panigrahi, comb. nov., based on *Aspenium arifolium* N. L. Burm. Fl. Ind. 231. 1768].

Ex *Hemionitide* L. sensu stricto (*H. levyi* Fourn. excepto) laminibus integris, laminis sterilibus ovato-cordatis et laminis fertilibus hastato-triangularibus, non lobatis differt; ex *Doryopteride* J. Smith laminis pilosis non glabris differt.

Rhizome erect or ascending, the scales linear, strongly bicolorous. Fronds numerous, simple, dimorphic, crowded on the rhizome; stipes stout, blackish, nitid, sparsely scaly above the base, the single vascular bundle U- to V-shaped; laminae chartaceous, dark green, the margins slightly revolute, with a prominent, black midrib and one pair of very short to long, basal primary veins sometimes visible on the abaxial surface of the laminae, the venation otherwise occult (but visible with transmitted light), areolate, the areolae polygonal, elongate, largest near the midrib, spreading outward toward the margin, lacking included veinlets. Sterile fronds several, shorter than the fertile ones and forming a rosette; stipes (1)4–10 cm long; laminae cordate or rarely hastate, with usually round basal lobes and a narrow sinus, often bearing buds at the base, sparsely pilose on the abaxial surface, the hairs obscurely jointed, tan. Fertile fronds 1 – few, longer than the sterile ones, erect; stipes (8)10–25(30) cm long; laminae hastate with pointed (rarely

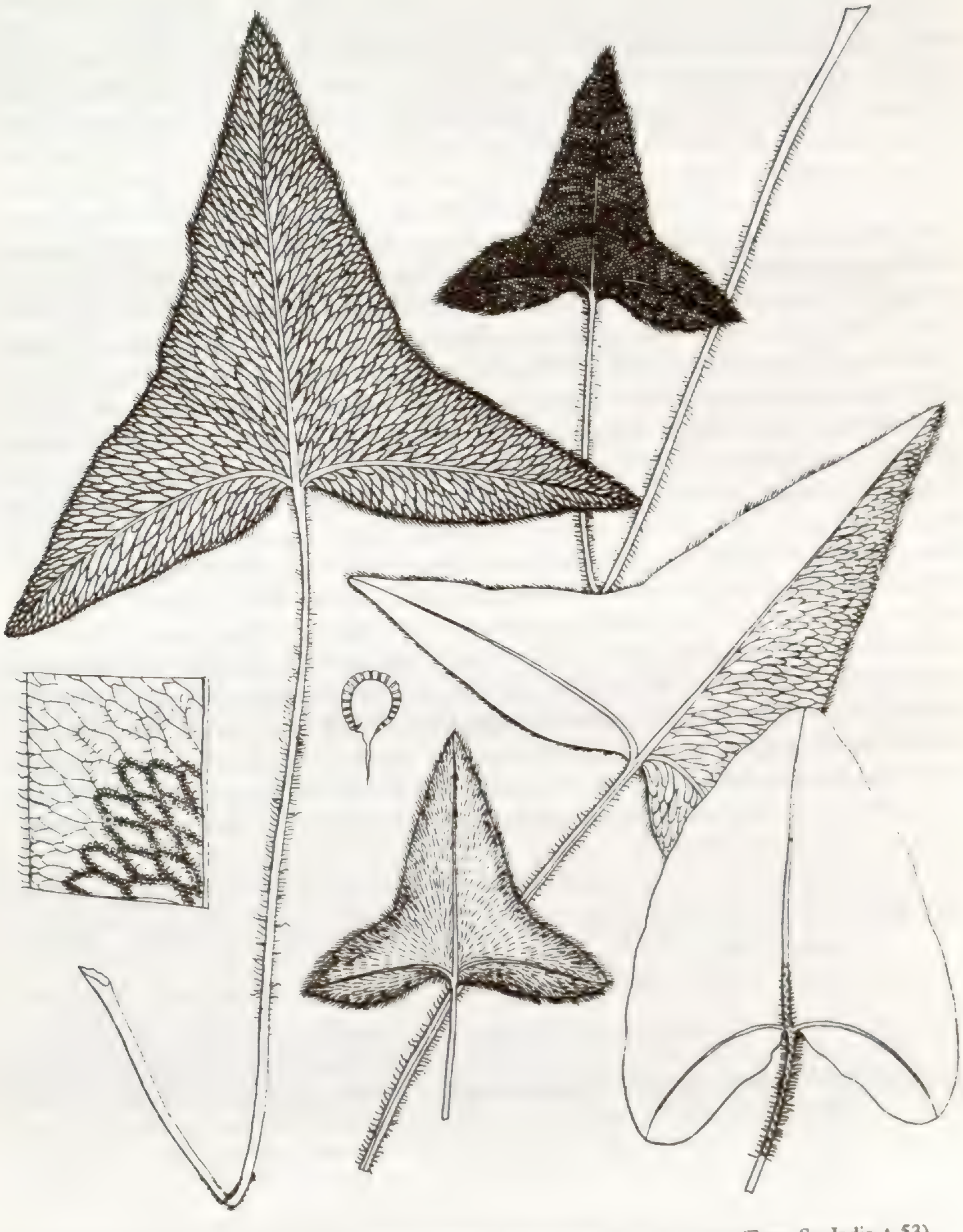

Fig. 1. *Parahemionitis arifolia* (N. L. Burm.) Panigrahi, reproduced from Beddome (Ferns So. India, t. 53).

round) basal lobes and a usually wide sinus, lacking buds at the base of the laminae, more densely pilose on the abaxial surface, the hairs obscurely jointed, pale tan; sporangia borne all along the veins, exindusiate; $2\underline{n} = \text{“}\underline{n}\text{”} = 90$ (obligate apogamous triploid) or 120 (obligate apogamous tetraploid).

Parahemionitis arifolia is distributed in India (South India to Orissa), Sri Lanka, Bangladesh, Burma, Indo-China, southern China, Malaya, and eastern Malaysia to the Philippines. The petiolar bulbils, which have been studied anatomically by Nicolas (1983, 1985), develop in India during October and November, rather profusely so in South India.

Among the species of *Hemionitis* L. sensu lato, *Parahemionitis* approaches only the Central American *H. levyi* Fourn., which has entire to 3-lobed sterile and fertile laminae and erect-ascending rhizomes. The related tropical American species *H. palmata* L. (lectotype of the genus) and *H. pinnatifida* Baker have short rhizomes, dimorphic fronds, and 5- or 7-lobed laminae. Other American species of the genus have 5- or 7-lobed or pinnate or bipinnate laminae, and some have creeping rhizomes.

Although *Parahemionitis* approaches some Asiatic species of *Doryopteris,* such as *D. ludens* (Wall. ex Hook.) J. Smith, in its sterile frond morphology, the rhizomes of these species tend to be long-creeping, the stipes scattered, the fertile fronds decidedly lobed, and the sporangia confined to the lamina margins and protected by pseudoindusia.

Parahemionitis has also been compared with *Paraceterach* Copel., a genus considered by Holttum in Airy Shaw (1966) to be monotypic and restricted to Queensland, Australia. Tryon (1987) later transferred several additional species to the genus. The hairs on the abaxial surface of the laminae of *Parahemionitis* differ greatly from the scales of *Paraceterach marantae* (L.) Tryon (syns. *Notholaena marantae* (L.) Desv. and *Cheilanthes marantae* (L.) Domin), which are broad, soft, and also are unlike the scales of *P. muelleri* (Hook.) Copel., which are stiff and bear marginal teeth of cilia.

Benham & Windham (1992, p. 55) concluded that the similarities between *Paraceterach* and their new genus *Astrolepis,* which includes the species of the *"Notholaena sinuata"* complex, were due to convergence, rather than to any close relationship. *Parahemionitis* differs from *Astrolepis,* as as it does from *Paraceterach,* in both lamina indument (hairs vs. scales) and in dissection (simple vs. pinnate). In addition, the base chromosome numbers differ in part in the three genera ($\underline{x}$ = 30 vs. 29 vs. 29 or 30).

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Observations on *Botrychium virginianum* (Ophioglossaceae), A Fern Uncommon in the Eastern Andes of Peru

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Botrychium virginianum (L.) Sw. is a widely distributed terrestrial herb in the New World, Europe, and Asia. In numerous humid, mid-latitude forests, this fern species has been described as frequent or common in occurrence (e.g., Tryon et al., 1940; Massey, 1944; Cranfill, 1980; Theiret, 1980; Lellinger, 1985; Cody & Britton, 1989). Fewer records are known for the low-latitude sites of the New World (e.g., Proctor, 1985; Mickel & Beitel, 1988; Lellinger, 1989) and observations on population sizes have not been published. Widespread fern species that are common in mid-latitudes and uncommon in low latitudes would form a biogeographical element of great interest, if this can be shown to be a general pattern and not due to lack of information from the tropical sites.

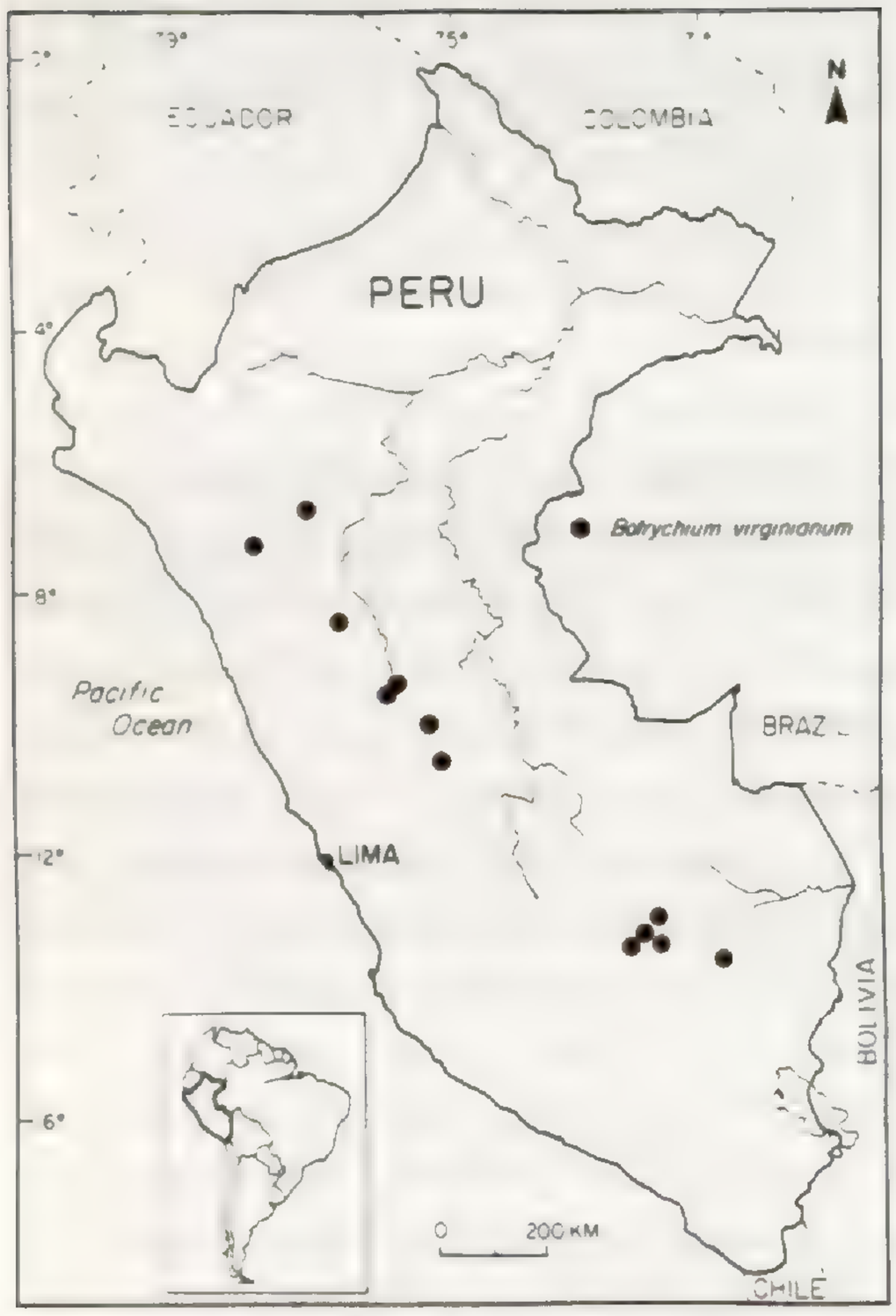
In Peru, *Botrychium virginianum* is currently known from 12 localities (Tryon & Stolze, 1989; our observations). These are primarily on the Eastern Cordillera of the Andes (Fig. 1), in the departments of Amazonas, Cajamarca, San Martín, Huánuco, Pasco, Junín, and Cusco, at elevations of 1800–3500 m, and from montane forest or scrub vegetation. Yet it was not the regional scarcity of *Botrychium virginianum* in Peru that first attracted our interest, but its local scarcity among the vegetation types we were studying in a national park in northern Peru (Young & León, 1988, 1990, 1991): of approximately 750 herbarium specimens made of pteridophytes from this park, only three were from *B. virginianum.* During the installation of a one hectare permanent tree plot in the park in June and July of 1988, we noticed this fern's presence within the demarcated area and decided to inventory it's sporophyte population.

In this study, we quantify *Botrychium virginianum's* presence on the forest plot, and then propose explanations for its local and regional distribution patterns.

STUDY AREA AND METHODS

The study area was in Chochos valley in the tropical subalpine zone of Rio Abiseo National Park, at approximately 3350 m elevation and 7°39'S, 77°29'W. Annual precipitation is probably at least 2500 mm, the mean annual temperature is approximately 5–8°C, and the climate is virtually aseasonal, with only a short dry season in July or August.

Young (1990: Chapter 3) chose a site within the timberline forest, but near the edge of that forest with high elevation grasslands (Fig. 2), and there he established a square one hectare plot that was gridded using stakes and meter tapes into 2500 contiguous 4-m^2 subplots. On each of these subplots, the identity, diameter, and height was recorded of every tree, shrub or tree fern with a minimum diameter of 2.5 cm at breast height (dbh). Each subplot was classified in terms of presence or absence of fallen trees or large branches, large boulders appearing above the soil surface, and canopy openings at least 1 m^2 in size and visible as projected vertically from a 2 m height (Brokaw, 1982). For *Botrychium virginianum,* we recorded the number of sporophytes in each subplot and the



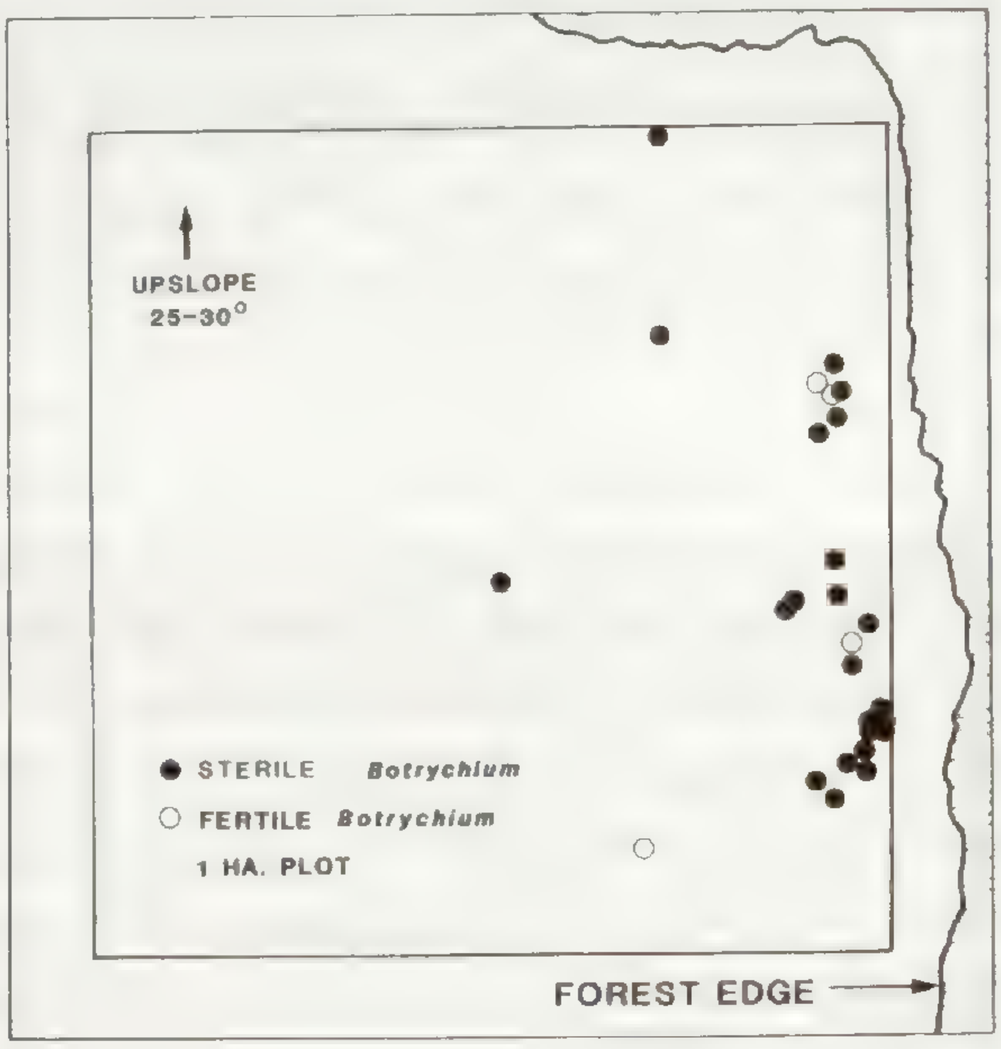


Fig. 1. Sites of herbarium collections of *Botrychium virginianum* in Peru, based on Tryon and Stolze (1989) and the authors' collections and observations.

Fig. 2. Locations of sporophytes of *Botrychium virginianum* on the 100 by 100 m plot made in a timberline forest bordering upon high elevation grasslands.

presence of any fertile leaves. Great care was taken during our fieldwork not to alter the understory by cutting or otherwise making trails, and no living plants were damaged or removed.

Young (1990: Chapter 3) described and analyzed the composition and abundance of the trees and shrubs on this plot, and offered some preliminary information on the tree ferns (Table 1). He found a significant positive association (Chi-square test, $p < 0.01$) between the presence of fallen trees or branches and the presence of canopy openings on the 4-m^2 subplots. Another noteworthy finding was a strong edge effect on the plot related to the presence of open subalpine grasslands 1–15 m from one of the plot's margins (Fig. 2).

Table 1. Site characteristics, and woody plant and tree fern diversity and abundance on the one ha forest plot sampled at 3350 m in the eastern Andes of Peru (Young, 1990).

Slope angles: 25– 30°.	
Soils: acidic, with 5-20 cm organic matter on top of 20–100 cm of a black silt loan derived from glacial loess.	
Trees and shrubs with diameters ≥2.5 cm:	
	46 species from 25 families and 32 genera.
	Consisted of 1801 stems, with 38.8 m^2 of basal area.
	Most common species were *Weinmannia auriculata* D. Don, Cunoniaceae;
	Hedyosmum scabrum (R. & P.) Solms, Chloranthaceae;
	Myrsine cf. *andina* (Mez) Pipoly, Myrsinaceae;
	Ilex sp., Aquifoliaceae; *Ruagea hirsuta* (C.DC) Harms, Meliaceae.
Tree ferns with diameters ≥2.5 cm:	
	Two species: *Cyathea pallescens* (Sodiro) Domin, *Sphaeropteris atahuallpa* R. Tryon.
	Consisted of 330 stems, with 3.1 m^2 of basal area.
Forest canopy height was 10 m (SD = 3).	
Of 2500 contiguous 4-m^2 subplots, 78 had canopy openings, 682 had fallen logs or branches, and	
	12 had boulders.

This edge effect consisted of a lower forest canopy (8 m, instead of 10–11 m inside the forest), increased tree and shrub density (from about 1500 stems/ha to more than 2500 stems/ha on the edge), and the presence of eleven tree and shrub species restricted to forest edge habitat. He hypothesized that this was due only in part to the present-day environmental gradient of increased light on the forest edge (see also Young, 1993); there was also evidence that forest within about 40 m of the edge was not in compositional equilibrium and was, in fact, in a late successional stage, probably recovering from a grassland fire that may have entered and devastated the stand of trees many years previously. The subalpine grasslands of the study area do not burn naturally. Instead, grassland burning is a common tradition by local people, as it is in similar areas elsewhere (Horn, 1989; Laegaard, 1992).

Results

Twenty-nine plants of *Botrychium virginianum* were found on the one ha plot (Fig. 2). Four of these plants were fertile at the time of sampling.

Habitat affinities of *B. virginianum* on the plot were examined at two different spatial scales: that of the original 4-m^2 subplots and of those subplots aggregated into 625 16-m^2 subplots. There were no significant positive or negative associations (Chi-square tests, $p = 0.58–0.90$), at either of these scales, between presence of the fern and presence of canopy openings, fallen logs or branches, and boulders. The only microhabitat affinity we were able to document was the presence of most (89%) of the ferns inside the forest but within 20 m of the subalpine grasslands (Fig. 2).

Discussion

Botrychium virginianum was present in the sampled forest at a density of 29 plants/ha. However, it was even less conspicuous than these numbers would indicate because of its small stature (10–25 cm) and its spatial clustering near the forest edge (Fig. 2). In continuous montane forest (i.e., without edges) there would presumably be little suitable habitat. The tests of association indicate that rock outcroppings or small canopy openings associated with tree- or branchfalls do not provide a suitable habitat for this fern. Even in our study area, where forest edges were fairly common, a pteridologist penetrating more than 20 m within the forest would be unlikely to find this fern.

Botrychium virginianum was not an edge species in our study area in the same sense as *Hypolepis obtusata*, which was found only in the 2–3 m wide zone of shrubby growth that separated the grassland from the forest (Young & León, 1991). Rather it was mostly found in forest that bordered on an open edge. There might be a set of environmental characteristics unique to this location that could explain its presence there. Or the critical factor for this fern might be related to the time elapsed following a disturbance, suggesting that its spatial distribution on the plot was due to an association with forest in a late successional stage. The latter explanation receives some support from a recent study done in central Sweden: Stahl (1990) showed that the largest populations of *Botrychium virginianum* were found in 5–30 year old conifer stands, while only very sparse populations were present in conifer stands 70–100 year old; new establishment of sporophytes was limited to those stands that were 5–15 year old.

Plant species that are specialists on habitat edges for whatever reason are naturally uncommon, at least as long as edges make up a small percentage of the area of a vegetated landscape. Add to that the inconspicuousness of *Botrychium virginianum*, and it is now clear why we had thought this fern to be rare in Rio Abiseo National Park.

Based on these observations, we suggest that *Botrychium virginianum* is probably much more ubiquitous in montane habitats in Peru than the herbarium collections would seem to indicate. However, due to its habitat requirements, this will be an uncommon fern everywhere except where forest edges are abundant and successional forests predominate. As these are the very features that characterize most of the humid temperate forests of North America and Europe (Curtis, 1959; Burgess & Sharpe, 1981; Naveh & Lieberman, 1984; Turner, 1987), it would appear that the relative abundance there of *Botrychium virginianum* is to be expected.

ACKNOWLEDGMENTS

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The Genetic Identification of Species of Agronomic *Azolla* Lam. Indigenous to Mexico

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The aquatic fern *Azolla* Lam. is familiar in tropical agriculture as a nitrogen biofertilizer in rice paddies of Southeast Asia (IRRI, 1987). Its symbiosis with the nitrogen-fixing cyanobacterium *Anabaena azollae* Strasb. exploits a renewable resource. Expansion of *Azolla* useage to other rice-growing regions is a realistic possibility when this fern is also utilized as animal or poultry feed, or in a tripartite rice-fish-*Azolla* system.

Agricultural application of *Azolla* is considered feasible in the neotropics. In Mexico, native *Azolla* species were found to grow rapidly, fix atmospheric nitrogen in significant amounts, and increase rice yield (Ferrera-Cerrato, 1980; Quintero Lizaola & Ferrera-Cerrato, 1987; Quintero Lizaola et al., 1990).

The difficulty of species identification, however, hinders the selection of desireable *Azolla* for field use. The morphological plasticity of New World taxa limits the utility of sporophytic characters for identification. Nonetheless, *A. filiculoides* (FI), *A. caroliniana* (CA), and *A. mexicana* (ME) have in the past been reported to be indigenous to Mexico (Svenson, 1944; Sota, 1976). Using molecular criteria, CA, ME, and *A. microphylla* (MI) are now known to be very genetically similar. They may be regarded as a "species complex", while FI is genetically distinct (Zimmerman et al., 1991a, b). The range of MI, when treated as a separate species, is believed to exclude Mexico (Schofield and Colinvaux, 1969).

This paper classifies and fingerprints *Azolla* collected in Mexico for agricultural purposes, using a diagnostic system of molecular markers. Growth results demonstrate the potential for this biofertilizer in rice agriculture of Mexico.

METHODS

Collection of Mexican Azolla. — The original 13 accessions of Mexican *Azolla* (Table 1) were deposited with the Centro de Edafologia del Colegio de Postgraduados (CEDAF). Most of this germplasm came from central and southern Mexico; *Azolla* from Sinaloa was the exception (Fig. 1). Some characterization was completed on newer accessions as they became available (Table 1). A number of these *Azolla* were obtained from the International Rice Research Institute (IRRI), which also possesses duplicates of various CEDAF accessions. Eight *Azolla* from IRRI had been tentatively identified as FI, CA, ME, or MI from morphological features.

Maintenance and Growth. — Fern sporophytes were maintained as clonal cultures at CEDAF under greenhouse conditions, and indoors at the University of Michigan-Dearborn. The latter environment included a mixture of natural sunlight and cool-white fluorescent lamps (12 h:12 h cycle) and a temperature of 25°C. Isolates were grown in N-free rice culture growth medium (Yoshida et al., 1971) in Mexico and N-free BG-11 medium (Rippka et al., 1979) in the USA.

Relative growth rates (RGR) of *Azolla* were estimated as changes in dry weights from

Table 1. Locale, coordinates, and altitude of each original *Azolla* collection site (01-13), and origins of newer accessions.

Key	Locale of collection	Latitude north			Longitude west			Altitude (m)
01 - CH	Chignahuapan, Puebla	19°	50'	16"	98°	00'	57"	2320
		19°	50'	44"	98°	01'	24"	
02 - JA	Cerro Gordo Mpio. de Emiliano Zapata, Veracruz.	19°	21'	50"	97°"	14'	20"	1435
03 - MO1	Atlacomulco, Mpio. de Jiutepec, Morelos	18°	46'	28"	99°	14'	47"	1350
04 - OA	Sta. Cruz. Mixtepeo, Mpio. de Zimatlán, Oaxaca	16°	48'	50"	96°	54'	12"	1500
05-SI1	Guamúchil, Sinaloa.	25°	26'	00"	108°	04'	32"	50
		25°	27'	13"	108°	05'	16"	
06 - TA1	Mazatiupa region, H.	17°	59'	30"	92°	26'	54"	10
	Cárdenas, Tabasco.	18°	05'	30"	92°	48'	35"	
07 - HI1	17 Km northwest of Ciudad	19°	53'	20"	98°	21'	54"	2520
	Sahagún in Laguna de	19°	50'	08"	98°	25'	44"	
	Tecocomulco, Hidalgo.							
08 - TE	Km 60, highway from	19°	17'	30"	98°	25'	45"	2300
	Mexico-Puebla, Texmelucan,							
	Tlaxcala	19°	18'	05"	98°	26'	30"	
09 - TL	Tláhuac, Distrito Federal.	19°	15'	25"	99°	02'	38"	2240
10 - TO	Sn. José Toshi, Edo. De	19°	51'	44"	99°	56'	20"	2550
	Mexico.	19°	52'	05"	99°	56'	38"	
11 - VI	Km 26.5, federal highway	19°	09'	50"	96°	15'	45"	18
	Veracruz-Xalapa, Mpio. de	19°	09'	58"	96°	15'	59"	
	Manlio Favio Altamirano,							
	Veracruz.							
12 - V2	Mpio. de Manlio Favio,	19°	21'	55"	97°	16'	10"	18
	Altamirano, Veracruz.							
13 - XO	Xochimilco, Distrito Federal.	19°	15'	10"	99°	05'	10"	2240
14 - MO2	Atlacomulco, Morelos	Site Date Unknown						
15 - TA2	Ceicades, H. Cardenas,	"						
	Tabasco							
16 - MO3	Atlacomulco, Mpio. de	"						
	Jiutepec, Morelos							
17 - MO4	Ticuman, Morelos	"						
1510 - HI2	Hidalgo	"						
2501 MC	Mexico City, Distrito Federal	"						
3510 TA3	Tabasco	"						
3519, 3520	Unspecified Collection Sites	"						
4506 - SI12	Sinaloa	"						
8001 - V3	Veracruz							
8031 - V4	Veracruz	"						

greenhouse cultures (Evans, 1972; Wong Fong Sang et al., 1987). Nitrogen accumulation was determined by RGR and %N (Bremner, 1965).

Fingerprinting

DNA polymorphisms. — Molecular evaluations were first conducted on the original 13 CEDAF accessions, which at the time composed all of the available Mexican *Azolla*. The procedures for harvesting and purifying DNA from *Azolla* have been described, as have the protocols for the analysis of DNA restriction fragment length polymorphisms (RFLPs) (Zimmerman et al., 1991b).

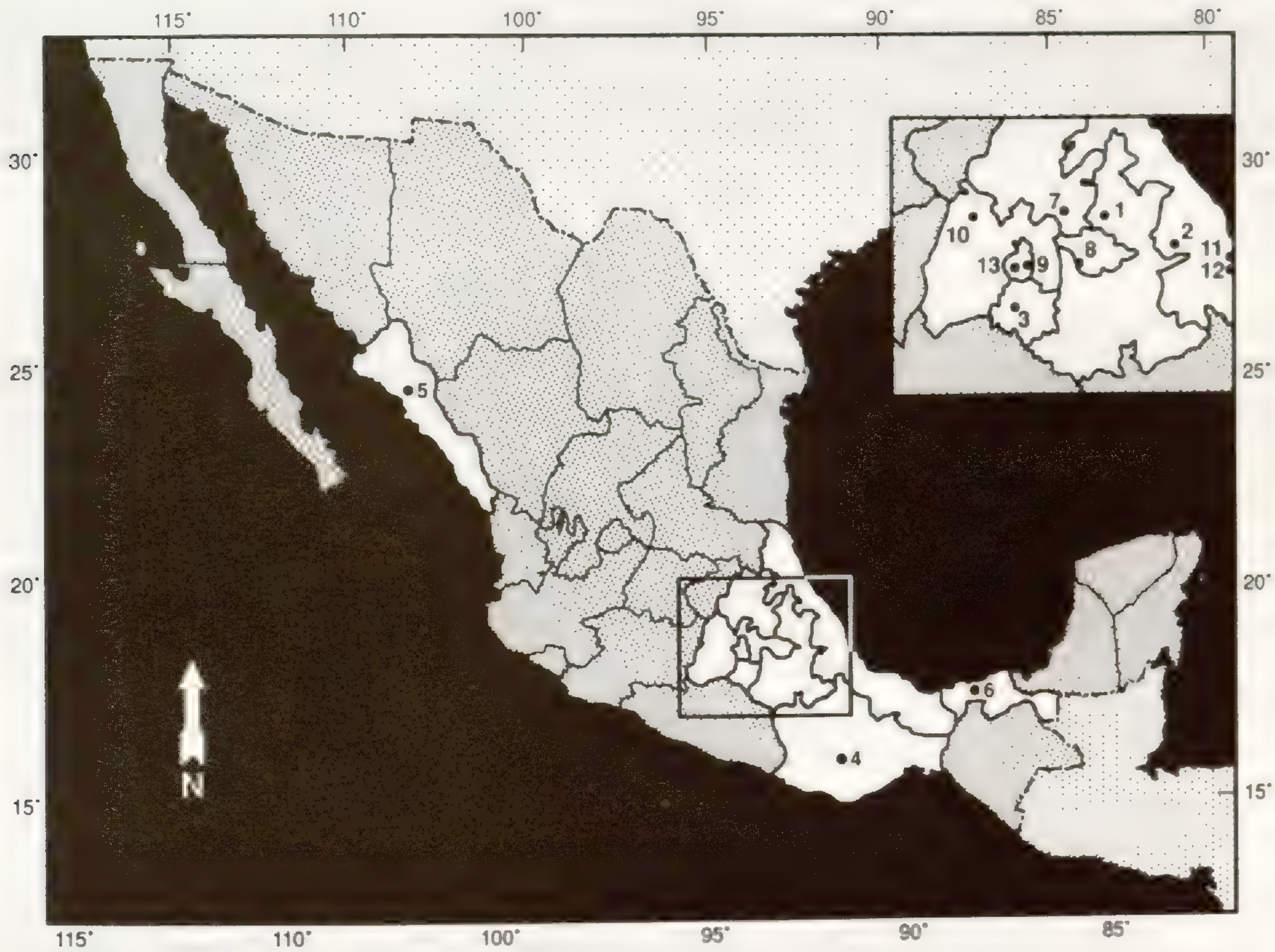

Fig. 1. Locales of *Azolla* collected in Mexico (see Table 1 for explanation of accessional code 01 - 13).

Two DNA probes were utilized in hybridizations. One was pA1HB3, a homologous random sequence of about 1.5 kb *Eco*RI ligated into pBlueScript, taken from a genomic library of *A. mexicana*. The other was pHA2, representing one pea genomic rDNA repeat unit, an 8.8 kb sequence in the *Hin*dIII site of pBR322 (Jorgensen et al., 1987).

Random amplified polymorphic DNAs (RAPDs) were obtained from all accessions by modifying a published protocol (Bassam et al., 1992). Approximately 25 ng of genomic DNA was used as template in 25 µl reactions containing 50 mM Tris HC1 pH 9.0, 5.0 mM $Mgcl_2$, 20 mM NH_4SO_3, 200 µM each dNTP, 1 µM primer, and 2.5 units *Tfl* DNA polymerase (Epicentre, Madison WI, USA). Primers (Genosys Biotech., Inc., The Woodlands TX, USA) used in this single-primer amplification technique were 10 bases in length with 50–80% G + C content.

Amplifications of DNA were performed on a MJ Research, Inc., Model 60 thermal controller (Watertown, MA). The amplification program included an initial denaturation of 1 min. at 94°C, then 35 cycles between 92°C and 30°C (1 sec. at each temperature), with a ramped heating rate of 1°C per 2 sec. Afterwards samples were given a 5 min. final elongation at 72°C, then held at 4°C overnight. Amplified DNA product was fractionated by electrophoresis in a 2% agarose gel (1% NuSieve GTG plus 1% Seakem LE; FMC Bioproducts, Rockland ME, USA) and stained with ethidium bromide for UV photography. Amplifications were always repeated at least twice to verify the consistency of results.

Allozymes. — Electrophoretic enzyme comparisons were conducted with all accessions.

Table 2. *Azolla* Lam. accessions of Mexico, original species classifications, and identification from molecular fingerprints (DNA and allozymes for 01 - 13; allozymes for the rest[a]).

EDAF No.	IRRI No.	Original Sp. Designation	Molecular Sp. Designation
01 - CH	–	–[b]	*filiculoides*
02 - JA	8028	–	CA/ME[c]
03 - MO	–	–	CA/ME
04 - OA	–	–	*filiculoides*
05 - SI	–	–	CA/ME (?) - unique
06 - TA	4139	microphylla	CA/ME
07 - HI	–	–	*filiculoides*
08 - TE	8030	–	CA/ME
09 - TL	–	–	*filiculoides*
10 - TO	–	–	*filiculoides*
11 - VI	–	–	CA/ME
12 - VII	3511	*caroliniana*	CA/ME
13 - XO	–	–	*filiculoides*
14	–	–	CA/ME
15	–	–	CA/ME
16	–	–	CA/ME
17	–	–	CA/ME
–	1510	*filiculoides*	CA/ME (?) - unique
–	2501	*mexicana*	*filiculoides*
–	3510	*caroliniana*	CA/ME
–	3519	*caroliniana*	CA/ME
–	3520	*caroliniana*	CA/ME
–	4506	*microphylla*	CA/ME (?) - unique
–	8001	–	CA/ME (?) - unique
–	8031	–	CA/ME

[a]Origins of newer accessions described in materials and methods.
[b]classified accession
[c]CA/ME = *caroliniana/mexicana*

Allozyme methodology was previously optimized for *Azolla* (Zimmerman et al., 1989a, b). Enzymes stained were glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49), isocitrate dehydrogenase (IDH; EC 1.1.1. 42), malate dehydrogenase (MDH; EC 1.1.1.37) malic enzyme (ME; EC 1.1.1.40), phosphoglucoisomerase (PGI; EC 5.3.1.9), phosphoglucomutase (PGM; EC 2.7.5.1), 6-phosphogluconate dehydrogenase (6PGD; EC 1.1.1.44), and shikimate dehydrogenase (SKDH; EC 1.1.1.25).

Results

The relative growth rates of the original 13 accessions under greenhouse conditions showed doubling times of wet-weight biomass from <4 days to >10 days. The putative FI accessions, as defined below, had six of the slowest seven growth rates and among the lowest N-accumulation rates. The most rapid RGR and N-accumulation was observed for 05-Sinaloa (2.17 g N g^{-1} dry weight y^{-1}), followed by 02-Jalapa (1.97 g N g^{-1} dry weight y^{-1}).

The results of RFLP, RAPD, and allozyme comparisons agreed in their assessments of *Azolla* species status (Table 2). The sole exception was 13-Xochimilco. The genetic fingerprints from DNA extractions at the start of this investigation unambiguously placed this accession with the FI group. However, zymograms conducted on biomass two years

later identified it as CA-ME. These results indicated that cross-contamination of that accession had occurred.

The other accessions identified as CA-ME included several with unique polymorphisms (05, 1510, 4506, 8001). Designated FI were 01, 04, 07, 09, 10, 13, and 2501. Corrections were made to the original classification of two accessions using the enzyme and DNA markers. *Azolla* 2501 – Mexico City had been inaccurately identified as ME and 1510 – Hidalgo as FI in the IRRI collection.

The pHA2 probe revealed RFLP polymorphisms within the ribosomal gene spacer regions of the original 13 CEDAF *Azolla*. The FI *Azolla* were distinguished by a band doublet at 1.2 kb in the 6-band ladder formed from hybridizations of pHA2 to *BamH*I-digested genomic DNA (Fig. 2). FI and CA-ME also differed in their patterns of middle repetitive DNA sequences obtained from genomic DNA digested with *EcoR*I or *EcoR*V and hybridized to pA1Hb3 (data not shown).

In *BamH*I-digested DNA, the pA1Hb3 probe elucidated a doublet of bands at approximately 5.5 kb and a faint band near 9kb for FI, compared to only a prominent single band at the 9-kb site for CA-ME accessions (Fig. 3). This separation was previously noted for *Azolla* accessions from other regions. The pA1Hb3 probe otherwise non-specifically hybridized to high molecular-weight DNA in the *BamH*I digest.

The RAPD fingerprints generated by a number of primers also defined *Azolla* accessions as FI or CA-ME. One example is primer CS-56 (Fig. 4); FI is identified by a major

Fig. 2. Restriction fragment length polymorphisms from a Southern blot of BamHI-digested *Azolla* genomic DNA hybridized to a pHA2 pea nuclear rDNA repeat unit. DNA size markers (kb) are located on the left margin. An asterisk marks each location of a hybrided doublet of bands. The order of accessions are: a) 01, b) 02, c) 05, d) 06, e) 07, f) 08, g) 09, h) 11, i) 12, j) 13, k) 04.

Table 3. Allozyme marker loci for designated FI and CA-ME accessions of *Azolla*. Those with unique electromorphic alleles are listed in parentheses.

Enzyme/Locus	FI Group[a]	CA/ME Group[b]
IDH	A	B
PGM-1	A[c]	B[c], (05/1510/4506)[c]
PGM-2	A (09)[d]	B (05/1510, 4506)
6PGD	A	B (06, 1510, 8001/4506)
SKDH-1	A	B (1510, 06, 8001)
SKDH-2	A (07)	B (05/06, 1510, 8001, 4506)

[a]01, 04, 07, 09, 10, 2501
[b]non-FI accessions (listed in Table 1)
[c]A = homozygous for fast allele; B = heterozygous; 5/1510/4506 = homozygous for slow allele of PGM-1
[d]A = heterozygous; 09 = homozygous for fast allele of A (differs in position from B group for PGM-2)

DNA doublet at about 915 and 875 base pairs, and CA-ME by bands at 900 and 715 bp (06-Tabasco yielded weakly amplified bands at these locations). Both taxa have a common band near 560 bp. This species dichotomy remained consistent with repeated amplifications, with template DNA from different extractions, and with *Azolla* controls from other regions.

Enzymatically, FI were identified by known allelomorphic markers (Table 3), including a slower PGM-2 locus and a faster IDH locus than those of other New World species (Zimmerman et al., 1989b). The IDH polymorphism assisted in the correction of the misidentified ME 2501 and FI 1510. Enzymes which did not show species specificity included the dimeric PGI (one locus visualized). Most *Azolla* were homozygous for the fast

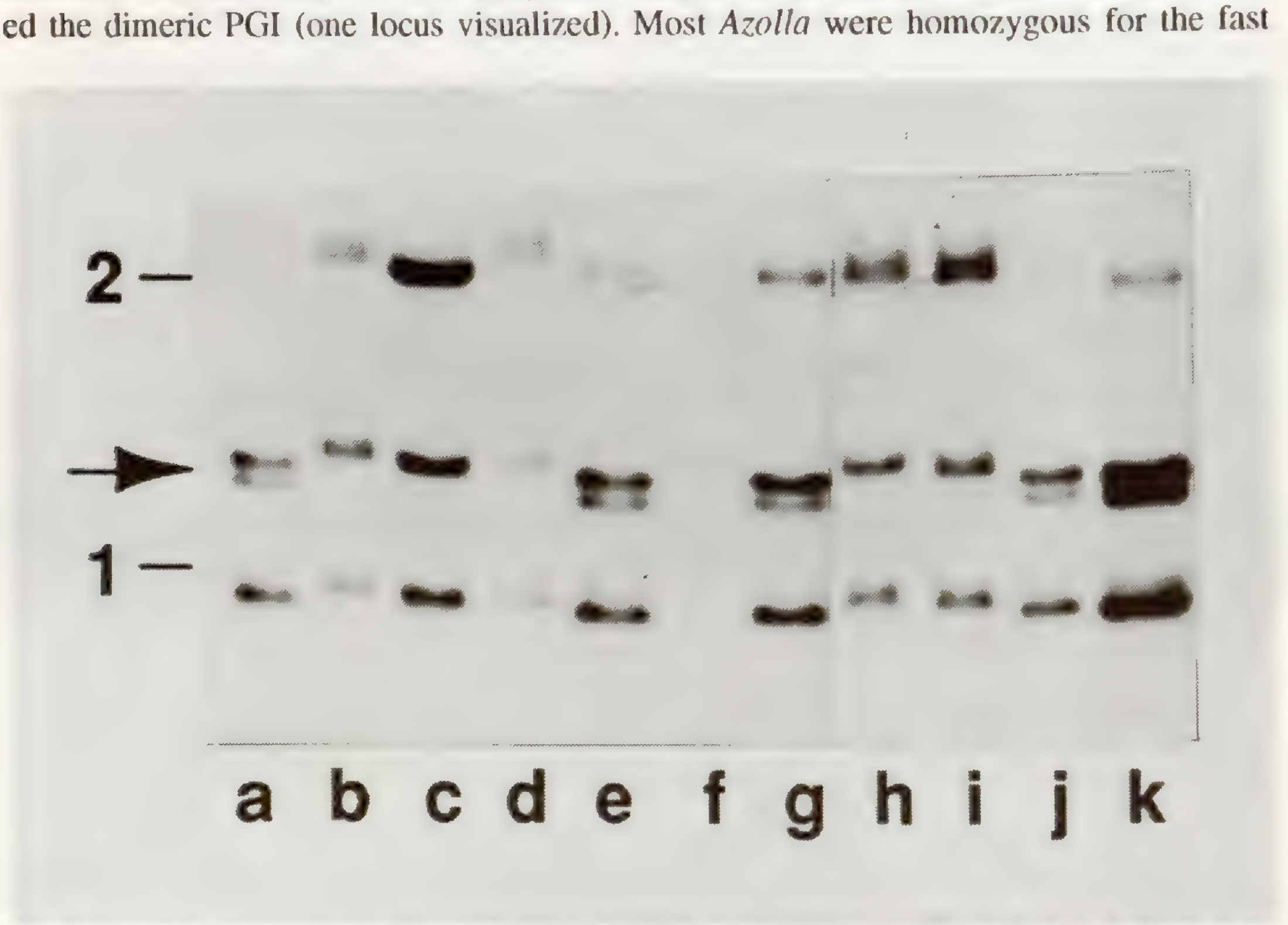

Fig. 3. Restriction fragment length polymorphisms from a hybridization of the Southern blot of Fig. 2 to the random homologous pA1Hb3 gene probe. A portion (3 of 6 bands) of the hybridization pattern is shown. An arrow indicates the location of the single or double bands which separate *Azolla* FI from CA-ME accessions. The same order of accessions are illustrated.

allele and only three were heterozygotes (1510, 4139, 13). The CA-ME *Azolla* were not as unified in their zymograms as the FI group, and individual accessions were at times polymorphic (Table 3). The status of 4139 and 4506 as MI was judged to be ambiguous from the molecular analyses. Finally, IRRI accessions 4506, 3510, and 1510 proved not be identical duplicates of CEDAF 05, 06, and 07, respectively.

Discussion

Accurate species identification of New World *Azolla* has always been an agronomic and botanical constraint. Diagnostic keys based on morphological features (Svenson, 1944; Lumpkin & Plucknett, 1982) are not reliable for most of these species. Likewise, compilation of unverified reports of *Azolla* occurrence, especially of tropical FI (Guatamala, Honduras, Colombia, Ecuador, Guyana, Trinidad), is of limited value in determining species ranges (Lumpkin & Plucknett, 1982).

Molecular fingerprinting is becoming essential in germplasm collections to check the identities (and thus the geographic origins) of *Azolla* accessions. Cluster analyses and distance phenograms from isoenzymes of the alleged taxa of *Azolla*, section Azolla, have circumscribed the CA-ME-MI group as well as the unique characters of FI and *A. rubra* (RU) (Zimmerman et al., 1989b). Direct genetic information from DNA fingerprinting has confirmed these observations (Zimmerman et al., 1991b).

This current investigation has now applied the appropriate markers to the working needs of agronomic botanists. An integrated phenetic-genetic system which identifies New World *Azolla* and the comprehensive profiling of an *Azolla* germplasm collection

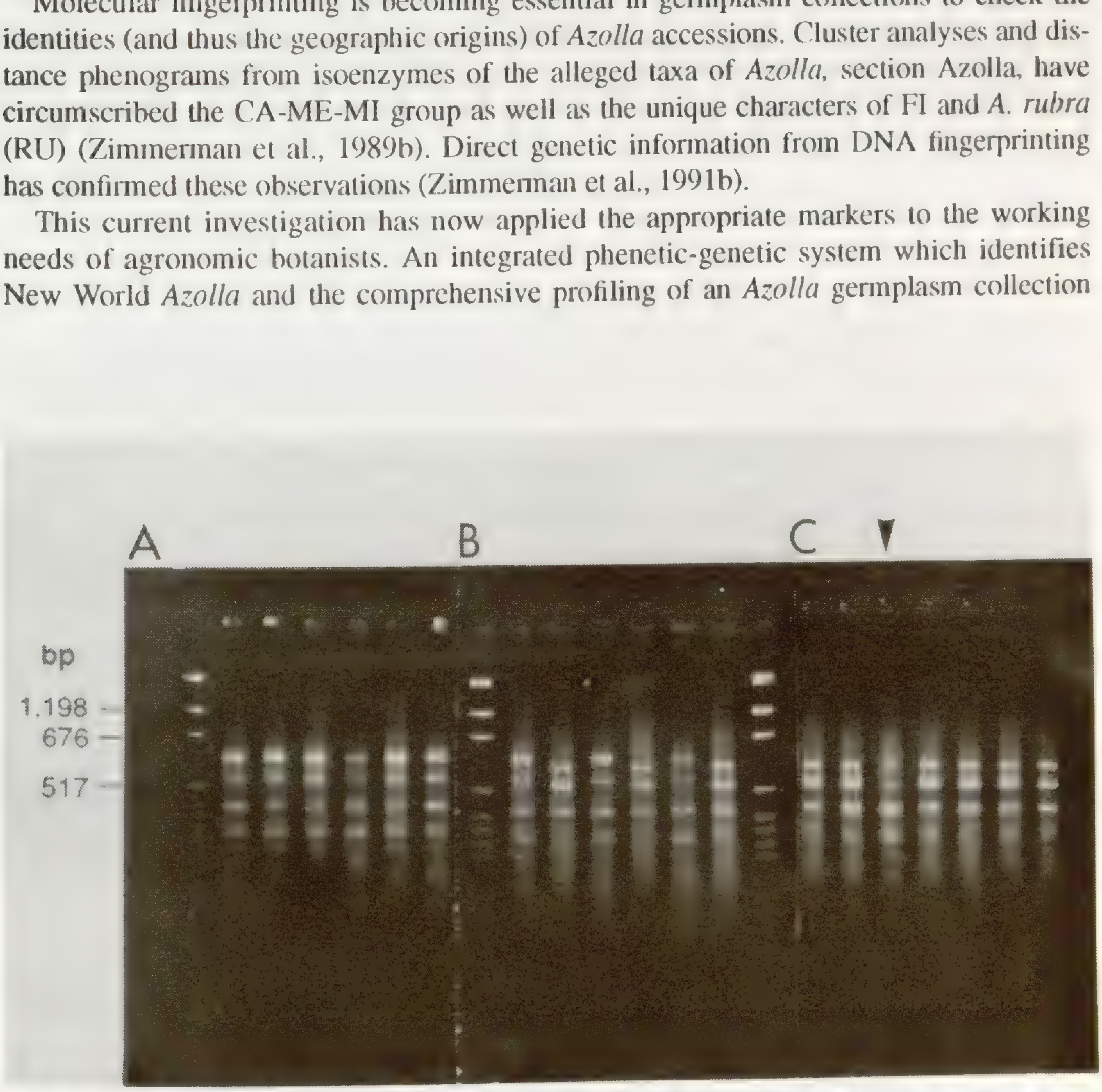

Fig. 4. Random amplified DNA polymorphisms separating *Azolla* FI from CA-ME accessions. The relevant primer was CS-56 (5' → 3' TGGTGGGTCC); reference DNA bands (base pairs) are seen in three lanes. FI accessions are located in Gel A, CA-ME in Gel C and both are compared in alternate lanes of Gel B. The arrow in Gel C indicates a slightly unusual RAPD with this primer for accession 06.

has been demonstrated for the first time. The latter objective is required to monitor cross-contamination and somaclonal mutation among the vegetative accessions.

The full distribution of *Azolla* species within Mexico is still not documented. In general, edaphic fern diversity is low except in elevated regions of the humid southeast (Palacios-Rios, 1991; Riba, 1991). Although several CEDAF *Azolla* accessions were collected in Veracruz, which is third in its species richness of ferns for all of Mexico (Palacios-Rios, 1991), one was also collected in species-poor Sinaloa. Since superior agronomic ecotypes may exist, collection of new *Azolla* of Mexico continues.

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The Genus *Isoëtes* L. in India

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The earliest reported Indian species, *I. coromandelina* was described by C. A. Linné, fil. in 1782 from the Coromandel coast, Tamil Nadu. For more than a century this remained the only species reported from India. Later workers found *I. coromandelina* in Karnataka, Kerala, West Bengal, Orissa, Bihar, Uttar Pradesh, Madhya Pradesh, and Gujarat. Mahabalé (1938) described a second species, *I. sahyadriensis* from a number of localities in the Western Ghats including Panchgani in Maharashtra and Kalahattigiri and Bababuddangiri and other peaks of Sahyadris in Karnataka. Soon thereafter, Rao (1944) and Shende (1945) described respectively, *I. sampathkumaranii* from Lalbagh Botanical Garden, Bangalore in Karnataka and *I. dixitei* from Panchgani in Maharashtra. These latter three reports of new species lacked detailed comparisons with previously described species. Subsequently, two more species, *I. panchananii* and *I. indica* were reported by Pant & Srivastava (1962). In their paper, Pant & Srivastava furnished detailed accounts and comparisons of their new species with all previously described Indian species. Recently five more species have been added to the list of Indian species. Two of these species were discovered in Madhya Pradesh. One species, *I. pantii* was described by Goswami & Arya (1970), and the other species *I. bilaspurensis* was described by Panigrahi (1981). The three remaining species *I. rajasthanensis, I. reticulata,* and *I. tuberculata* were described by Gena & Bharadwaja (1984). Two subspecies, *I. coromandelina* subsp. *coromandelina* and subsp. *brachyglossa,* were distinguished by Panigrahi (1981) on the basis of a reexamination of the herbarium specimens in the Kew Herbarium and British Museum (Natural History), London. A third subspecies, *I. coromandelina* subsp. *macrotuberculata,* was distinguished by Marsden (1976) from northern Australia. The eleven species from India is a relatively small number when compared to an estimated 400 to 500 species of *Isoëtes* world wide (personal communication Dr. H. P. Fuchs-Eckert). This low number of Indian species suggested a need for more extensive and intensive exploration for *Isoëtes* in various parts of India. For example no species of *Isoëtes* have been reported from the Himalayas, a region where *Isoëtes* should be found. Our recent explorations have yielded two species of *Isoëtes* which have not been previously described from peninsular India.

MATERIAL AND METHODS

Preliminary observations on fresh material were documented in the field. Living plants and specimens fixed in FAA were also studied in the laboratory at Allahabad. In the diagnoses and descriptions which follow the upper portion of the leaf is described as a limb and the flattened basal portion bearing the adaxial sporangium is described as a sheath. Spores were treated with HF, washed with water, and macerated with Schulze's fluid to separate different wall layers. Microspores were also acetolysed according to the method of Erdtman (1952). SEM photomicrographs were taken with a JEOL SEM model 35 C after sputter coating the dry untreated spores with a layer of gold-palladium approximately 200 Å thick. The descriptive terms used in the present paper follow the terminology of Jackson (1928), Kremp (1965), and Hickey (1986).

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NEW SPECIES

Isoëtes mahadevensis Srivastava, Pant & Shukla, sp. nov. (Figs. 1; 2, C, E; 3, A-D; 4, A-G; 6, C-D.). — TYPE: India, State of Madhya Pradesh, Distr. Hoshangabad, Pachmarhi, Chhote Mahadev; leg. G. K. Srivastava, no. 15011A, 1987, CAL; isotypes K, no. 15011B and Duthie hb. in Bot. Dept., Allahabad Univ. no. 15011C.

Planta subaquatilis; cormus bulbosus bilobatus vel nonnunquam trilobatus; et usque ad 15 mm latum; radices basi 0.2–1.2 mm crassae, folia 5–29, usque ad 14 cm longa, medio folio 1.0–1.5 mm lata, linearia, aliquantum extrorse incurvata, parallelimarginalia vel paululum undulata, parte distali in apicem longam subulatam fastigata, duae partes apicales folii stomatibus ornabae, vagna basalis applanata lutescente-subalbida usque ad 9 mm lata, unicum sporangium medianum adaxialem, (sive microsporangia in foliis exterioribus, sive megasporangia in foliis interioribus) portans, fibia fiberolignosa absentes; ligula trianguloidea, basi bilobata, 1.0–1.4 mm. lata et 0.9–1.3 mm longa, subfusca; labium triangulare, apice obtuso, albido-subflavidum, 0.43–0.65 mm latum et 0.30–0.5 mm longum; velum rudimentarium tholiforme, tegens sporangium 200 μm circiter in parte superiore et 170–500 μm ambobus lateribus solum; sporangia ovoidea, parte superiore applanata, 2–2.5 mm. lata et 3–4.5 mm longa; megasporae triletae obscure-griseae statu umescente, subalbidae statu sicco, dimorphae, i.e., duabus dimensionibus disparibus, maiores pyramidali-globosae, 384 μm–507 μm ($\bar{x}$= 415 μm), paries megasporarum quadritabulata, latere distali perisporii reticulato, lacunis completis vel interruptis, disjunctis inter se parietibus aut tam altis quam latis, aut latioribus altitudine selliformi, ornamentatione latere distali perspicue evoluta per costam aequatorialem, sed obscure aliis locis, latere proximali jugis triletis, rectus, tenuibus, 30 μm–48 μm altis, superficiebus pyramidalibus rugulis vel pustulis ornatis, costa aequatoriali undulata, tenue, usqua ad 17 μm alta; megasporae minores 203 μm–372 μm ($\bar{x}$ = 297 μm), paululum applanatae, ornamento ei megasporarum maiorum simili. sed facie dostali indisticto, superficiebus pyramidalibus latere proximali pustulis distantibus, secundum micrographes microscopii electronici cibrialis [i.e., Scanning Electron Microscope] superficie integra perisporii protecta fibris gelatinosis siliceis, interjectis, reticulum irregulare formantibus, saepe terminis singularibus solutis fractisque intra lacunas; exosporium atque mesosporium tenuiter granulata, firme conjuncta inter se, endosporium subtile, translucens, laevigatum, circumscriptione orbiculare; microsporae generatim monoletae, rarius triletae, rotundae vel subrotundae, 10 μm–47 μm ($\bar{x}$ = 33 μm), costa proximali recta, tenui, membranacea, costa laesurali 10 μm–30 μm longa atque usque ad 9 μm alta, pariete microsporii tritabulata, perisporio echinato secundum imagines microscopii luminaris [= LM] idem secundum micrographes microscopii electronici cibrialis [= SEM], exosporio subtiliter pustuloso, mesosporio minute granuloso.

Plant submerged; corm bulbous usually bilobate, rarely trilobate and up to 15 mm wide; roots 0.2–1.2 mm thick at base; leaves 5 to 29, up to 14 cm long, 1.0 to 1.5 mm wide at midlength, linear, slightly bent, outwards, with parallel or undulating edges, tapering into a long attenuate apex, apical two thirds of leaf limb stomatiferous, flattened basal sheath up to 9 mm wide, yellowish-white, bearing a single median adaxial sporangium (microsporangium in outer and megasporangium in inner leaves), peripheral strands absent; ligule triangular, with lobed base, 1.0 mm–1.4 mm wide, 0.9 mm–1.3 mm long, brownish-yellow; labium triangular with obtuse apex, yellowish-white, 0.43 mm–0.65 mm wide and 0.30 mm–0.50 mm long; velum rudimentary, shaped like a dome, covering about 200 μm of sporangial apex and 170 μm–500 μm of its two sides; sporangia ovoid, flattened at the upper side, 2–2.5 mm wide and 3–4.5 mm long; megaspores trilete, dark grey when wet, white in

dry condition, dimorphic, the larger ones pyramidal-globose, 384 μm–507 μm ($\bar{x}$ = 415 μm), spore wall four layered, perispore retate distally, lacunae complete or broken, separated from one another by muri, thickness of muri (tangential width) almost equal to or greater their anticlinal height, ornamentation on distal face developed clearly along girdle, but abscure elsewhere, proximal face with straight, thin, trilete ridges 30–48 μm high, interradial contact areas with small rugulae or pustules, equatorial ridge undulating, thin, up to 17 μm high; smaller megaspores 203 μm–372 μm ($\bar{x}$ = 297 μm), somewhat flattened, perisporal ornamentation similar to larger megaspores, but indistinct on distal face, interradial contact areas on proximal face with scattered pustules, in SEM photomicrographs the entire surface of perispore is covered by siliceous gel-fibres, interconnected with one another to form an irregular network, often with loose, broken ends within lacunae; exospore and mesospore finely granulate, firmly attached to each other, mesospore sometimes with numerous folds, edospore thin, translucent, smooth with circular, outline; microspores generally monolete, rarely trilete, round to roundly oval, 10 μm–47 μm ($\bar{x}$ = 33 μm), proximal ridge straight, thin and membranous, 10 μm -30 μm in length and up to 9 μm high, spore wall three layered, perispore under LM and SEM echinate, exospore finely pustulate, and mesospore finely granular.

PARATYPE: India: State of Madhya Pradesh, Kasdol Distr. of Raipur: Kasdol Jungal, along water streams; leg G.K. Srivastava, No. 15021, 1987, Duthie hb. in Botany Dept., Allahabad Univ.

Plants of this species grow luxuriantly in small, shallow ditches either fully or partially submerged in water (pH = 5.4) and the soil is sandy and loamy. The siliceous gel-fibers mentioned in the diagnosis are formed by silica deposited in the form of a gel (Tryon & Tryon, 1982).

On the basis of megaspore ornamentation the retate (reticulate) megaspores of *I. mahadevensis* come closest to those of *I. panchananii, I. reticulata,* and *I. panchganiensis* whose megaspores are reticulate. The ornamentation of megaspores of *I. mahadevensis,* however, differs from that of the megaspores of the above mentioned species in having muri whose breadth and height are almost equal (the muri in other species are considerably higher than broad). The rugulate megaspores of *I. rajasthanensis* are also comparable with those of *I. mahadevensis,* but their muri too are higher than broad. In addition *I. rajasthanensis* differs from *I. mahadevensis* in having reportedly smooth microspores, whereas those of *I. mahadevensis* are echinate and unusually globose. The rudimentary velum of *I. mahadevensis* distinguishes it clearly from other Indian species belonging to the section *Terrestres* A. Br. [= *Reticulatae* Pfeiffer]. The velum in the other Indian species either covers nearly the entire sporangium or half of it as in *I. panchananii.*

The specific name "mahadevensis" chosen and derived from the type locality of the species "Chhote Mahadev."

Isoëtes panchganiensis Srivastava, Pant, and Shukla, sp. nov. (Figs. 2, A, B, D; 5, A-F; 6, A, B.). — TYPE: India, State of Maharashtra, Dist. Satara, Panchgani; leg. G. K. Srivastava, no. 15001A, 1987, CAL; isotypes: K. no. 15001B and Duthie hb. in Bot. Dept. Allahabad Univ. no. 15001C.

Planta subaquatilis; cormus trilobatus, diametro usque ad 12.0 mm; radices basi 0.2–0.9 mm crassis; folia 9–20, 7–13 cm longa, medio folio 1.2–3.0 mm lata, linearia, minute extrorse incurvata parallelimarginalia vel paululum undulata, parte distali in apicem longam spiculiformem fastigata, viridia duae partes folii, stomatibus ornatae, dilatata, vagina basalis applanata, lutescente-subalbida, usque ad 7 mm lata, unicum sporangium medianum adaxialem portantia; ligula trianguloidea, basi lobii dilatata, 1.2 mm–2.1 mm lata et 0.7–1.5 mm longa, albido-flavida; labium triangulare, 0.5 mm–1.2 mm latum et 0.5–0.7 mm longum; velum hyalinum, sporangium fere omnino tegens, basi solum fenestram ovali-elongatum, 1.2 mm latam et 0.8 mm altam reliquens; sporangia ovoidea, parte superiore applanata, 2.5–3.0 mm lata et 3.0–4.0 mm longa; megasporae triletae, griseae

Fig. 1. Holotype of *Isoëtes mahadevensis* No. 1501A. Bar = 1 cm.

statu umescente, albidae statu sicco, dimorphae, i.e., maiores pyramidali-globosae, 270 μm–456 μm ($\bar{x}$ = 364 μm), paries megasporarum quadritabulata, latere distali perisporii reticulato, lacunis generatim completis, disjunctis parietibus inter se, altitudine selliformi alteriore latitudine, costa aequatoriali sine reticulationibus, facie proximali jugis triletis, rectis, tenuibus, 35 μm–50 μm altis, superficiebus pyramidalibus lacunis absentibus costa

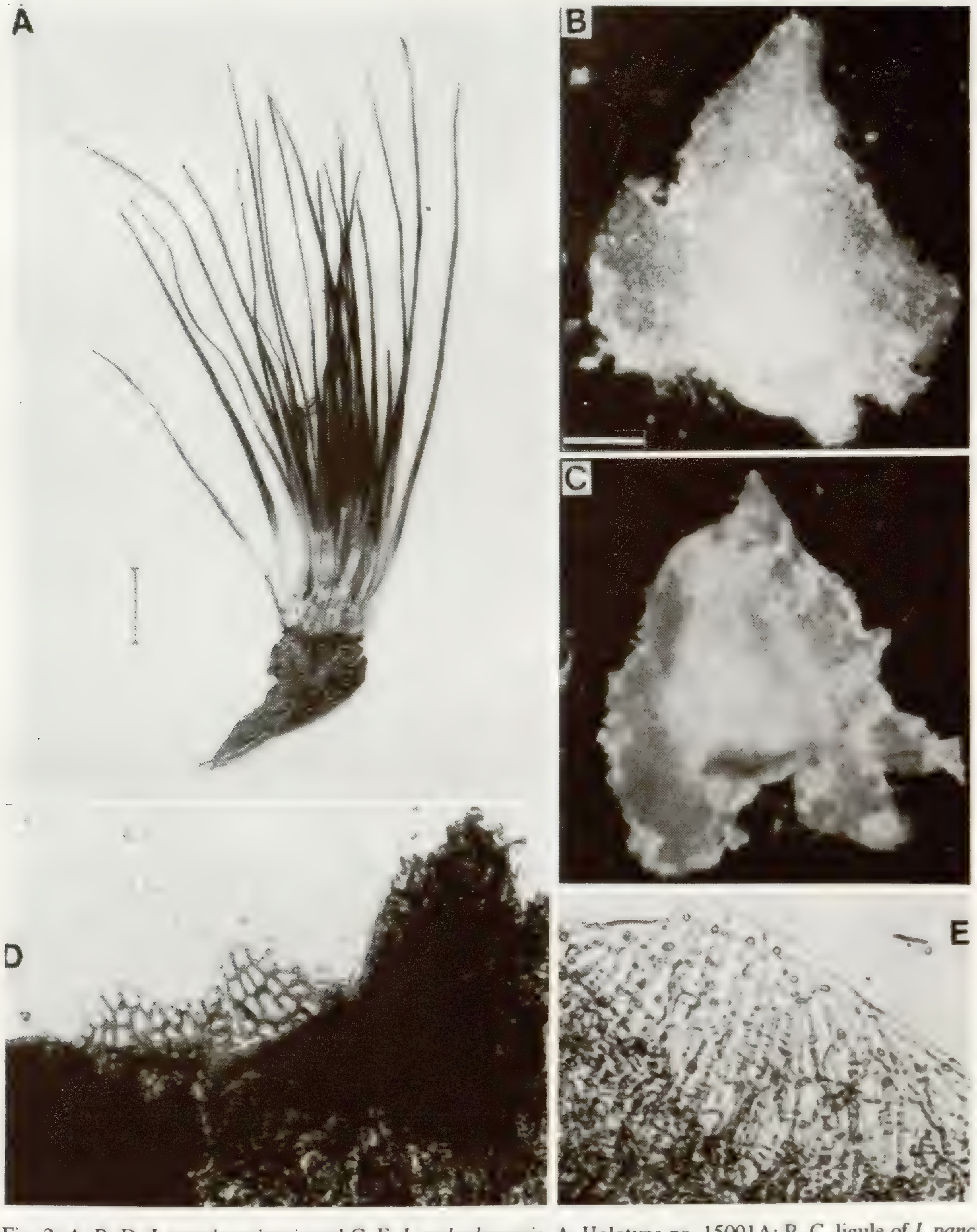

Fig. 2. A, B, D, *I. panchganiensis* and C, E, *I. mahadevensis*. A, Holotype no. 15001A; B, C, ligule of *I. panchganiensis* and *I. mahadevensis;* D, E, labium of *I. panchganiensis* and *I. mahadevensis* respectively. Bar in A = 1 cm; B, C = 185 μm; D = 58 μm and E = 25 μm.

aequatoriali leviter undulata, 10 μm–17 μm alta; megasporae minores 185 μm–305 μm ($\bar{x}$ = 244 μm), paululum applanatae, ornamento ei megasporarum majorum simili, sed facie distali indistincto, superficiebus pyramidalibus unico vel duabus pustulis, secundum micrographes microscopii electronici cibrialis [= SEM], superficie universa perisporii protecta fibris gelatinosis siliceis, nonnunquam interconnectis inter se, sed plerumque ter-

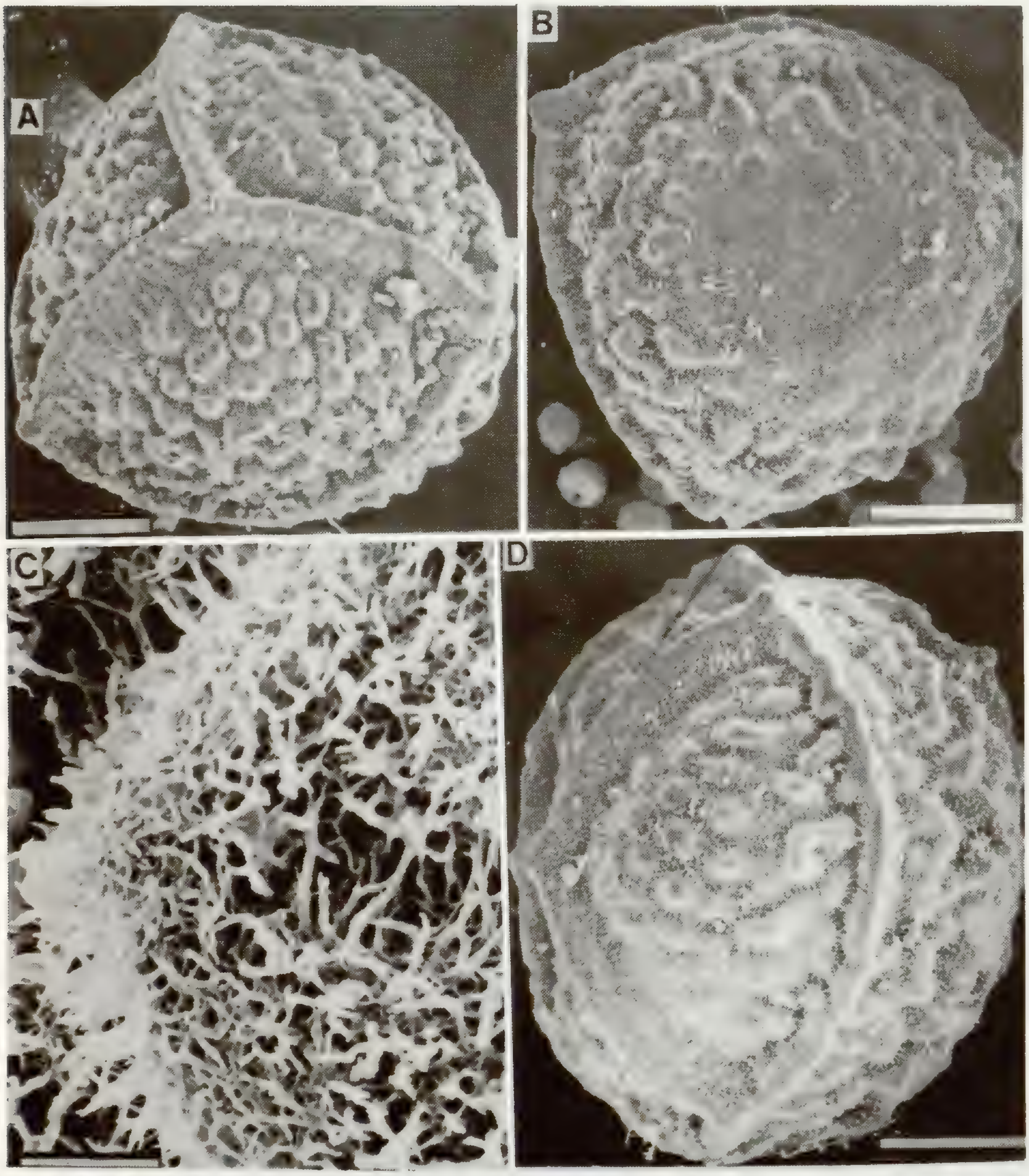

Fig. 3. A-D, SEM photomicrographs of megaspores of *I. mahadevensis*. A, proximal view; B, distal view; C, a portion of Fig. 3 B further magnified to show infrastructure details of the surface; D, lateral view. Bars in A, B, D = 100 µm, and C = 10 µm.

minis interruptis, formantibus imaginem irregularem retiformem in lacunis; exosporium et mesosporium tenuiter granulata, firme conjuncta inter se, mesosporium numerosis plicis superficialibus, endosporium subtile, translucens, laevigatum, circumscriptione orbiculare; microsporiae ignotae.

Plant submerged; corm bulbous trilobate up to 12.0 mm in diameter; roots 0.2 mm–0.9 mm thick at base; leaves 9–20, 7–13 cm long, 1.2 mm–3.0 mm wide at mid-length, linear, slightly bent, outwards, with parallel or slightly undulating edges, tapering into a long, attenuate apex, apical two thirds of leaf limb stomatiferous, limb green, flattened basal sheath up to 7 mm wide, yellowish-white, bearing a single median adaxial sporangium; ligule triangular, with expanded base, 1.2 mm–2.1 mm wide, and 0.7 mm–1.5 mm long yellowish-white; labium triangular, 0.5 mm–1.2 mm wide, 0.5 mm–0.7 mm long; velum hyaline, covering nearly the entire sporangium

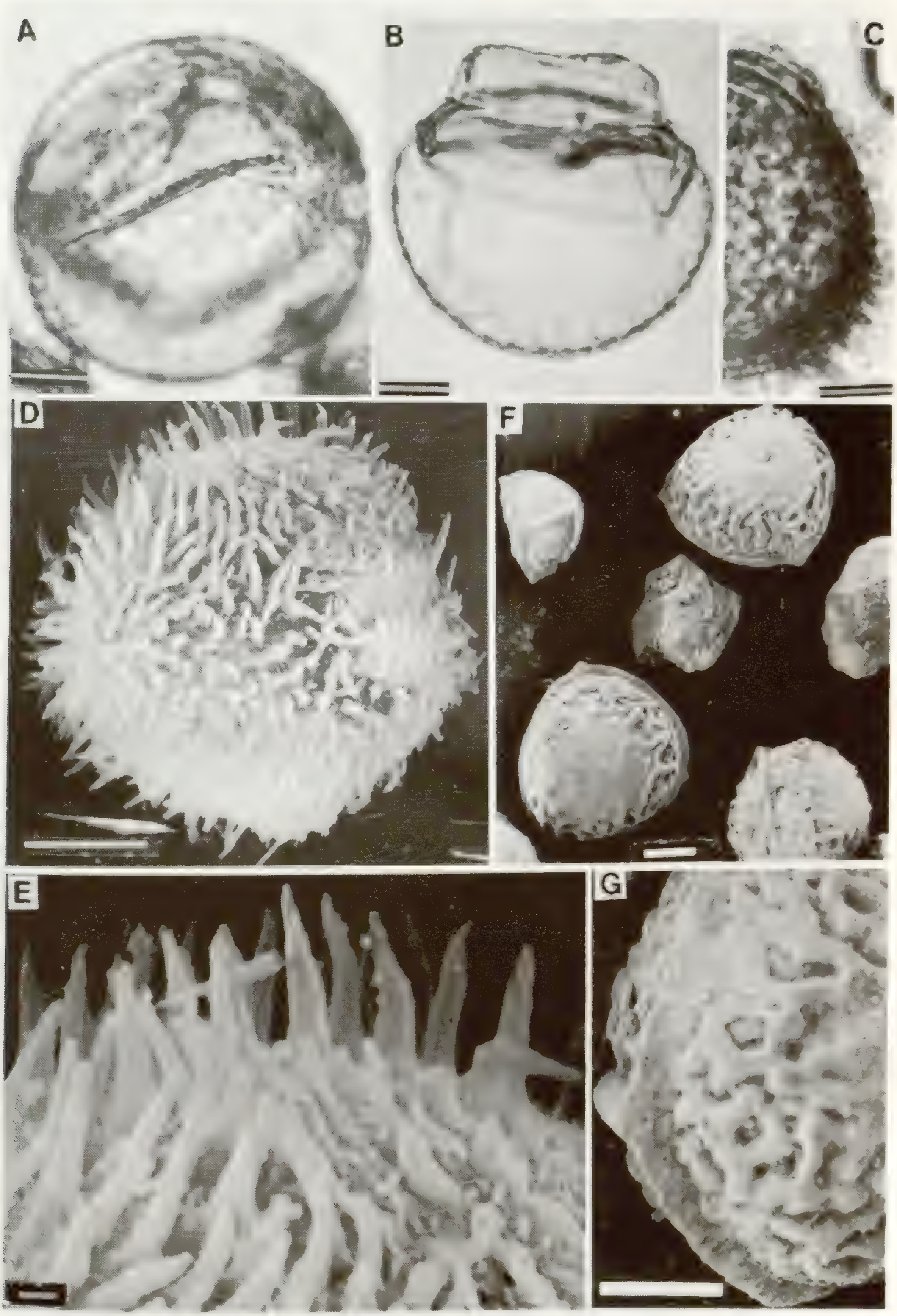

Fig. 4. A-C (LM) and D, E (SEM) photomicrographs of microspores and F, G (SEM) photomicrographs of megaspores of *I. mahadevensis*. A, proximal view; B, lateral view; C, a portion of margin showing spines; D, lateral view; E, a portion in Fig. 4 D further magnified to show the details of spines; F, three large and one small megaspores lying in different angles; G, a portion of large megaspore in Fig. 4 F further magnified to show details of the surface. Bars in A-C = 8 μm; D = 10 μm; E = 1 μm; F, G = 100 μm.

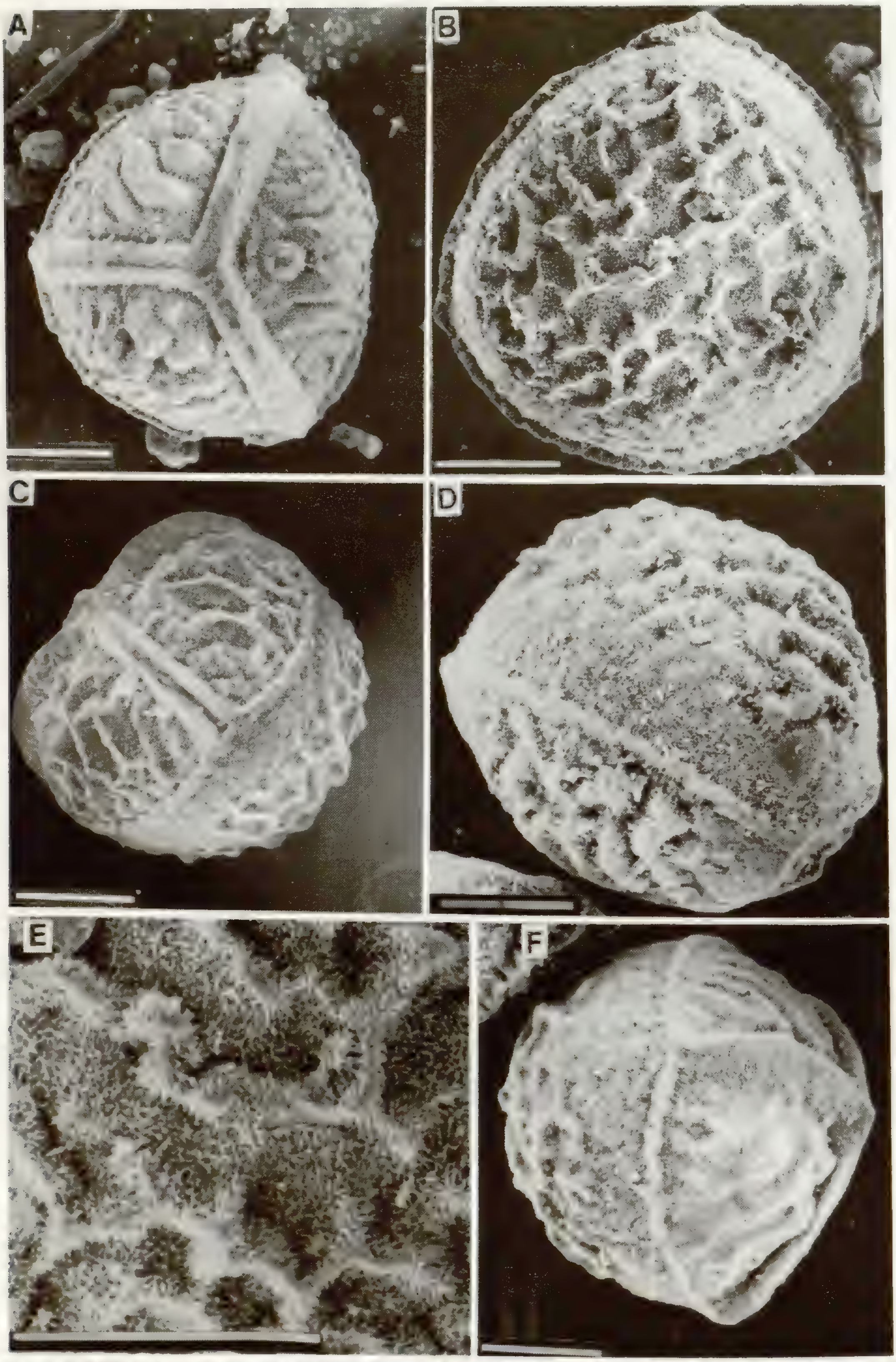

Fig. 5. A-F SEM photomicrographs of megaspores of *I. panchganiensis*. A, proximal view; B, distal view, C, D, F, lateral views; E, a portion of Fig. 5 B further magnified to show infrastructure details of the surface. Bars in A-F = 100 μm.

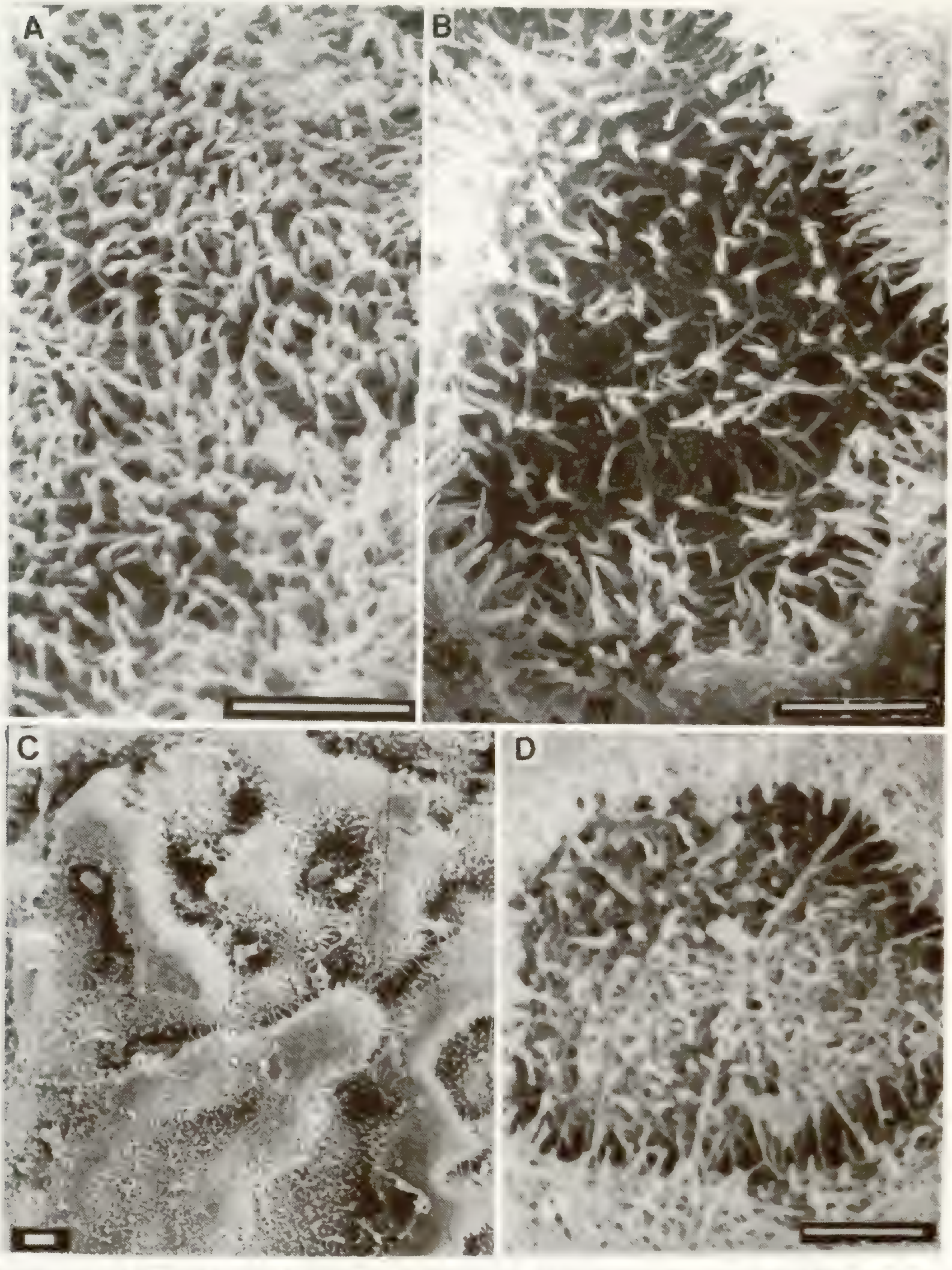

Fig. 6. A, B SEM photomicrographs of portions of megaspores of *I. panchganiensis*, and C, D of *I. mahadevensis* magnified to show infrastructure details of their surfaces. Bars in A-D = 10 μm.

except for a small ovally-elongated, 1.2 mm broad, and 0.8 mm high window at base; sporangia ovoid, flattened at upper end, 2.5–3.0 mm wide, and 3–4 mm long; megaspores trilete, grey when wet, white when dry, dimorphic, larger ones, pyramidal-globose, 270 μm–456 μm (x = 364 μm), spore wall four layered, perispore reticulate distally, lacunae generally complete, separated from one another by muri, anticlinal height of muri greater than thickness of muri (tangential width), girdles without reticulations, proximal face with straight, thin trilete ridges 35 μm–50 μm high, interradial contact areas without lacunae, equatorial ridge slightly undulating 10

µm–17 µm high; smaller megaspores 185 µm–305 µm ($\bar{x}$ = 244 µm), somewhat flattened, perisporal ornamentation similar to that of larger megaspores but indistinct on distal face, interradial contact areas on proximal face with one or two pustules, in SEM photomicrograph entire surface of perispore is covered by siliceous gel-fibres, sometimes interconnected with one another but usually having broken ends forming an irregular net-like pattern in lacunae; exospore and mesospore minutely granulate, firmly attached to each other, mesospore with numerous surface folds, endospore thin, translucent, smooth, with circular outline; microspores unknown.

Isoëtes panchganiensis var. **kemangundiensis** Srivastava, Pant, & Shukla var. nov. (Figs. 5 C, D, F). — TYPE: India, State of Karnataka, Kemangundi, Chikmanglur Distr. leg G. K. Srivastava no. 15031A, 1987 CAL; isotypes: K no. 15031B; and Duthie hb. in Bot. Dept. Allahabad Univ. no. 15031C.

Megasporae areolis incompletis et minute majoris; megasporae majores 322 µm–564 µm ($\bar{x}$ = 408 µm); megaspores minores 209 µm–306 µm ($\bar{x}$ = 250 µm).

Megaspores with incomplete areoles and slightly larger; larger megaspores measure 322 µm–564 µm ($\bar{x}$ = 408 µm) and smaller megaspores measure 209 µm–306 µm ($\bar{x}$ = 250 µm).

Plants of *I. panchganiensis* grow submerged and their luxurious growth often forms a mat in the water of small shallow ditches together with *I. dixitei*. The soil is clay and silty loam, and the pH of water is 5.7.

Two varieties of *I. panchganiensis* sp. nov. are distinguished. The type of the species *I. panchganiensis* var. *panchganiensis* shows megaspores which are slightly smaller and usually show complete areoles. The second variety, *I. panchganiensis* var. *kemangundiensis* var. nov. shows larger megaspores with incomplete areoles. The two varieties are assigned to the same species since they are otherwise identical.

The varietal name "kemangundiensis" is derived from the type locality of the variety "Kemangundi".

A unique feature of this species is a differentiated girdle on the distal face of megaspores adjacent to the equatorial ridge which is devoid of reticulations. No other Indian species shows this feature.

I. panchganiensis is closest to *I. panchananii* in having almost the same type of reticulate magaspore ornamentation. *I. panchganiensis,* however, can be distinguished from *I. panchananii* by several differing characters. First, the velum of *I. panchganiensis* almost completely covers the sporangium whereas the velum of *I. panchananii* covers about half of the sporangium. Second, the reticulate ornamentation of megaspores of *I. panchganiensis* is confined to the central region of the distal face whereas the reticulate ornamentation covers the entire distal face of the megaspores of *I. panchananii* including the girdle. Third, the megaspores of *I. panchganiensis* are slightly larger than those of *I. panchananii.* Furthermore, mature plants of *I. panchganiensis* are generally smaller than those of *I. panchananii.* In addition the sporangial wall of *I. panchganiensis* does not show thick-walled cells like those present in the sporangial wall of *I. panchananii.* SEM photomicrographs of megaspores of *I. panchganiensis* show relatively thicker siliceous gel-fibres. They do not form connected networks and their broken ends appear as peg-like structures while the siliceous gel-fibres of megaspores of *I. panchananii* form a network over the muri and lumina of the reticulations. *I. panchganiensis* is also comparable to *I. reticulata* and *I. rajasthanensis* in having a complete velum, with only a basal slit. The characteristic smooth girdle of *I. panchganiensis,* however, distinguishes it clearly from them as do the differences in the pattern of siliceous gel-fibres. In *I. panchganiensis* siliceous gel-fibres are often interconnect with one another and form an irregular network while in *I. reticulata* they do not interconnect with one another.

An aberrant plant having a velum almost completely covering the sporangium had been mentioned by Pant & Srivastava (1962) as having been collected form the same locality, Panchgani. Since these authors had only one plant, they failed to recognize it as a new species and assigned it tentatively to *I. panchananii,* but this can now be referred to the present species.

The specific name "panchganiensis" is derived from the type locality of the species "Panchgani."

Quillwort Flora of India

The present study raises the number of Indian species of *Isoëtes* to thirteen whose distribution is depicted in Fig. 7. An artificial key for the identification of these species is provided at the end of this paper. Indian species of *Isoëtes* can be assigned to different sections of infrageneric (supra specific) classification system in *Isoëtes* which was given by Pfeiffer (1922) and later modified by Fuchs (1962, 1981). Of these, seven species, *I. coromandelina, I sahyadriensis, I. dixitei, I. indica, I. pantii, I. bilaspurensis,* and *I. tuberculata* have tuberculate or pustulate megaspores and are therefore assignable to be the section *Palustres* [A. BRAUN, in mss., ex] GRENIER in GRENIER & GODRON (1856), emend. FUCHS (1982) [= sectio *Tuberculatae* PFEIFFER (1922)]. The only species which can be assigned to the section *Isoëtes* [= Sectio Cristatae PFEIFFER (1922)] is *I. sampathkumaranii* since Pant & Srivastava (1962) had clearly demonstrated that it showed at some places confluent pustule bases forming small ridges on the distal face of the megaspores (see P1.13, figs. 5–8). Although Rao (1944) had mentioned that the megaspores of this species showed "upper segments marked with reticulate branched ridges." The remaining five species, viz., *I. panchananii, I. reticulata, I. rajasthanensis, I. panchganiensis,* and *I. mahadevensis* show reticulations on the distal megaspore faces and are thus attributable to the section *Terrestres* [A. BRAUN in mss., ex.] GRENIER in GRENIER & GODRON (1856), emend. FUCHS (1982) [= Sectio *Reticulatae* PFEIFFER (1922)]. Presently no Indian species is assignable to the sections *Echinatae* Pfeiffer (1922) and *Laeves* Fuchs (1981).

As far as the species of the section *Palustres* are concerned, the proximal as well as the distal faces of megaspores show essentially similar ornamentation, but the megaspores of other Indian species belonging to sections *Isoëtes* and *Terrestres,* show typical sculpturing of the respective sections only on the distal faces of the larger megaspores, the proximal faces of larger as well as smaller megaspores of these species are atypical. In *I. sampathkumaranii* the distal faces of the megaspores show reticulate branched ridges as described by Rao (1944) but their proximal faces bear pustules or small ridges. Similarly distal faces of megaspores of *I. rajasthanensis* show rugulae but their proximal faces have pustules or small rugulae. The megaspores of *I. panchananii* and *I. panchganiensis* show distinct areoles formed by muri on the distal faces but the muri never form areoles on their proximal faces. Earlier workers (Boom, 1980; Duthie, 1929; Hickey, 1986; Proctor, 1949; Williams, 1943) have already mentioned that the proximal and the distal faces of megaspores may show different ornamentation. Hickey (1986) has in particular mentioned that 'it has generally been assumed that the distal spore face represents the "official" character state and it is on the basis of its ornamentation alone that species have been classified.' He further points out that "a species like *I. eshbaughii* could be assigned to either sect. *Isoëtes* or *Palustres* on the basis of distal spore morphology, whereas using the proximal ornamentation it could be included only in sect. *Palustres.*" We would, how-

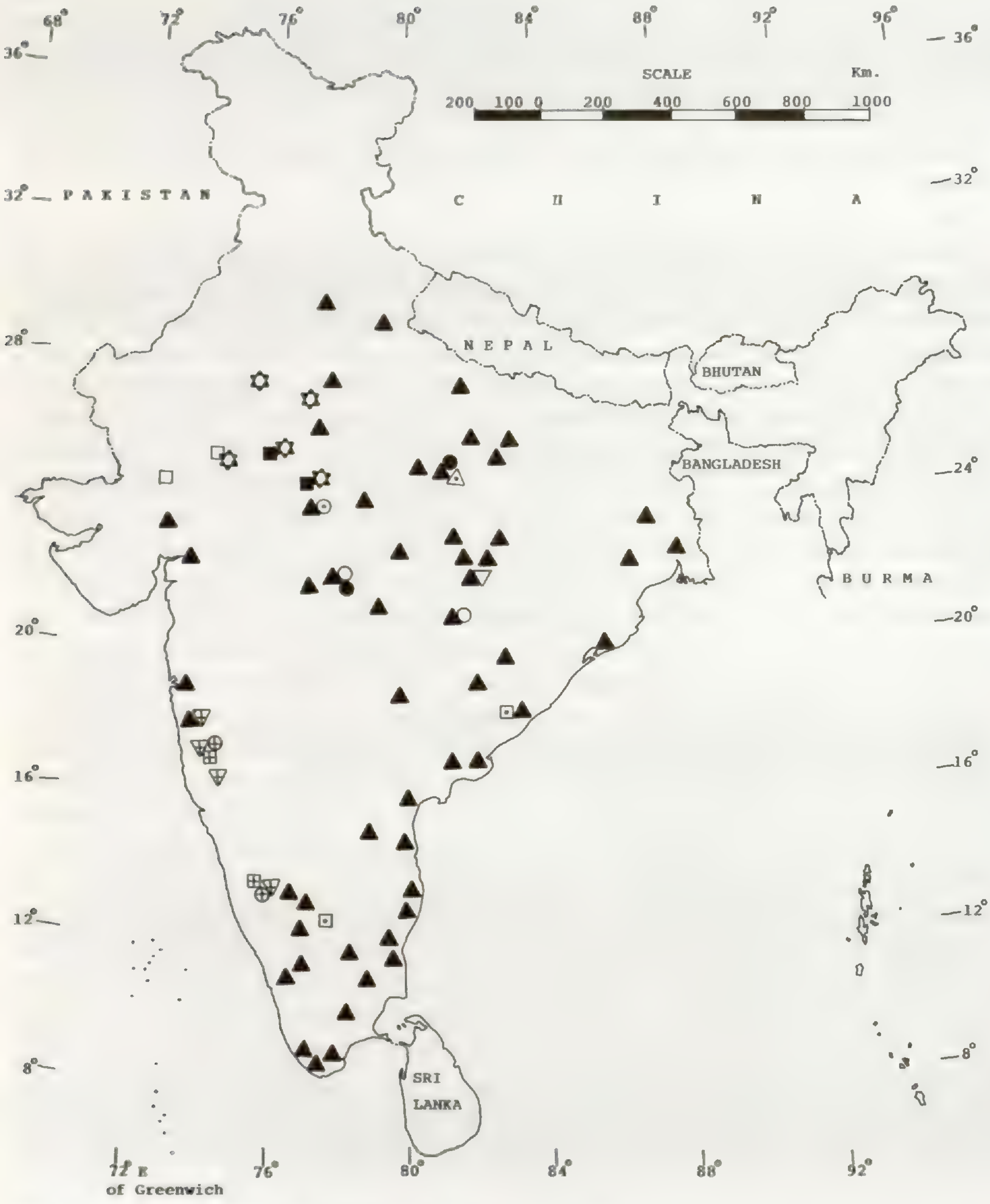

Fig. 7. Map of India depicting the geographical distribution of difference species of *Isoëtes*. Open triangle = *I. bilaspurensis;* Closed triangle = *I. coromandelina;* Cross in triangle = *I. dixitei;* Dot in triangle = *I. indica;* Open circle = *I. mahadevensis;* Closed circle = *I. panchananii;* Cross in circle = *I. panchganiensis;* Dot in circle = *I. pantii;* Open square = *I. rajasthanensis;* Closed square = *I. reticulata;* Cross in square = *I. sahyadriensis;* Dot in square = *I. sampathkumaranii;* Star = *I. tuberculata*..

ever, suggest that in classifications based on megaspore morphology it will be better if we consider the ornamentation on both faces instead of single face morphology since it is expected that we would arrive at a more natural classification by taking into account all the available characters. So far as megaspores are concerned, if it becomes necessary we

should be prepared to increase the numbers of sections of *Isoëtes* to accomodate forms with differing proximal-distal face ornamentation of larger megaspores as well as the differing ornamentation of smaller megaspores.

The occurrence of two kinds of megaspores seems to be a general feature of all Indian species of the genus, since this is so not only in almost all previously described species, but also in the two new ones described here. *I. sahyadriensis* may be the only exception, but the account given by Mahabalé (1938) lacks necessary details and the type is not traceable; in spite of several attempts to collect duplicate material, we were unable to find the species in the type locality.

A review of the literature shows that megaspore dimorphism is quite prevalent even in species of *Isoëtes* described from Africa (Braun, 1868; Pfeiffer, 1922; Duthie, 1929; Alston, 1956; Reed & Verdcourt, 1956; Wanntorp, 1970; Hall, 1971) and Australia (Marsden, 1976). Perhaps there is a need to reexamine all species of the genus to confirm the presence or absence of this character.

The factors responsible for the prevalence of dimorphism of megaspores of *Isoëtes* are not definitely known. Irregular meiosis observed in many species, particularly the Indian ones, could, however, be the cause since that has been observed in *I. coromandelina* (Abraham & Ninan, 1958; Verma, 1961) and *I. indica, I. panchananii* and *I. pantii* (Pant & Srivastava, 1965; Goswami, 1975). In this connection, Ninan (1958) has observed that "in the megaspore mother cells of *I. coromandelina* there is no chromosome reduction during `meiosis' and consequently megaspores with the unreduced number of chromosomes are formed" and such observations have also been confirmed in the other three species. However, Marsden (1976a) has suggested that polyploidy and apomixis may be the possible causes of polymorphism of megaspores in *I. muelleri* A. Br. Further work is necessary to establish the factors responsible for dimorphism of spores in different species of the genus.

The altitudinal distribution of the species of *Isoëtes* in India shows that taxa having pustulate or tuberculate megaspores show a wide altitudinal range in their distribution. A species like *I. coromandelina* occurs from the near sea level to fairly high hilly regions (up to 1075 m). Even amongst such species, however, *I. dixitei* and *I. sahyadriensis* are confined to the hills from 1350 m to 1923 m. On the contrary, the reticulate spored species like *I. panchananii, I. rajasthanensis, I. reticulata, I. mahadevensis,* and *I. panchganiensis* are confined only to higher altitudes (from 287 m – 1923 m).

Key for the Identification of the Indian Representatives of *Isoëtes* L.

1. Megaspores: tuberculate or pustulate
   [Section *Palustres* (= *Tuberculatae)*]
   2. Sporangia described as microsporangia containing mixed small spores (microspores 15–54 μm in diameter) and a few large megaspores about 200 μm in diameter
      I. pantii
   2. Megasporangia containing only di- or trimorphic megaspores
      3. Megaspores tuberculate, tubercles with pointed apices and of almost uniform size
         I. indica

3. Megaspores pustulate, pustules with rounded apices

4. Pustules of almost uniform size

5. Velum present covering 2/3 of megasporangia

I. bilaspurensis

5. Velum absent

6. Megaspores dimorphic

I. coromandelina

6. Megaspores trimorphic

I. tuberculata

4. Pustules of distinctly heterogenous size

7. Velum complete having only a slit-like aperture at the base

I. sahyadriensis

7. Velum rudimentary

I. dixitei

1. Megaspores: Reticulate, retate, rugulate
[Section *Terrestres* (= Reticulatae)]

8. Anticlinal height of muri almost equal to or less than tangential thickness of muri, velum rudimentary

I. mahadevensis

8. Anticlinal height of muri greater than tangential thickness of muri

9. Velum covering more than 3/4 of the sporangium

10. Reticulations present only on central part of distal face, absent in girdles

I. panchganiensis

10. Reticulations present all over the distal face

11. Megaspores dimorphic

I. reticulata

11. Megaspores trimorphic

I. rajasthanensis

9. Velum covering 1/2 - 3/4 of sporangium

12. Muri generally forming areoles

I. panchananii

12. Muri in the form of very short cristae rarely forming areoles

I. sampathkumaranii

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Morphological Studies on Entomogenous Stem Galls of *Microgramma squamulosa* (Kauf.) Sota (Polypodiaceae)

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The cells, tissues and organs of many plants are subject to the effects of a series of factors which may result in pathological growth, characterized by disease and loss of function or atypical growth, which involves changes in normal patterns of development and differentiation (Bloch, 1965; Rohfritsch & Shorthouse, 1982). One of the more complex forms of atypical growth is that known as gall. Galls are structures which originate through processes of hypertrophy and hyperplasia, inhibition of growth or cytohistological modification, in response to the attacks of gall-inducing organisms (Mani, 1964).

More than 15,000 types of gall are at present known; they are found on algae, fungi, lichens, bryophytes, pteridophytes, gymnosperms and angiosperms (Mani, 1964). Gall inducers are also widely varied, and include algae, lichens, fungi, bacteria, viruses, nematodes, arachnids and insects. The principal cause of gall formation is insects, and of the groups known to participate in this phenomenon, the most important in terms of frequency and importance are Coleoptera, Hemiptera, Diptera and Lepidoptera (Mani, 1964; Occhioni, 1979; Fernandes & Martins, 1985).

Fossil evidence of various levels of interaction between pteridophytes and the arthropods dates back to the Devonian period, and in some cases suggests complex associations (Scott *et al.*, 1985). Even so, entomogenous galls are not frequently found among cryptogams (Houard, 1933; Mani, 1964; Fernandes, 1987). Interactions between insects and pteridophytes have been studied by Mohan-Daniel & Chandrasekar (1986). According to Fernandes (1987), of the principal types of gall-inducing agents only insects are important for pteridophytes. Mani (1964) claims that the family Polypodiaceae, comparatively young and rich in number of species, is the best represented in terms of numbers of galls. The same author points out that none of the galls which occur in this family are known to be caused by Lepidoptera.

This work is concerned with some morphological aspects of entomogenous galls found on the stems of *Microgramma squamulosa,* an epiphytic Brazilian fern, and with the understanding of the inter-relationship between plant and inducing agent.

MATERIALS AND METHODS

The plant material under investigation is the epiphytic fern *Microgramma squamulosa* (Kaulf.) Sota (Polypodiaceae), which grows on trees of *Tipuana tipu* Benth. (Fabaceae), used as ornamentals in streets in the suburb of Bela Vista, City of São Paulo, Brazil. Aerial stems, both with and without galls, were collected and packed in damp closed plastic bags for purposes of transport. Analysis of the external morphology was based on

observation of the form of the galls, of their size at various stages of development, and of their position on the plant.

Stem segments with galls and unaffected segments were both used for anatomical studies. The samples were cut in pieces, put into Petri dishes containing damp filter-paper, and then transferred to FAA (formalin: acetic acid: ethyl alcohol 50° GL, 1:1:18, v/v) in accordance with Johansen (1940). After fixing, samples for study by light microscopy were dehydrated in an ethyl alcohol series and embedded in paraffin wax, as described by Johansen (1940) and Sass (1951). Serial transverse and longitudinal sections were then obtained and stained with Safranin and Fast Green (Moore, 1936). Some of the samples, after fixing, were dehydrated in a ketone series, and embedded in glycol-metacrylate (Reichert-Jung Historesin) as described by Beryln & Miksche (1976). Transverse and longitudinal sections were stained with Toluidine Blue (O'Brien *et al.*, 1964). Samples for electron microscopy were obtained after fixing, dehydrated in a ketone series, dried at critical point with CO_2, and prepared with gold and palladium as described by O'Brien & McCully (1981). Observation was carried out at 15–20 Kv. The most important aspects of morphology were recorded through photomicrographs and electromicrographs. Drawings were made with the help of a camera lucida attached to a stereomicroscope.

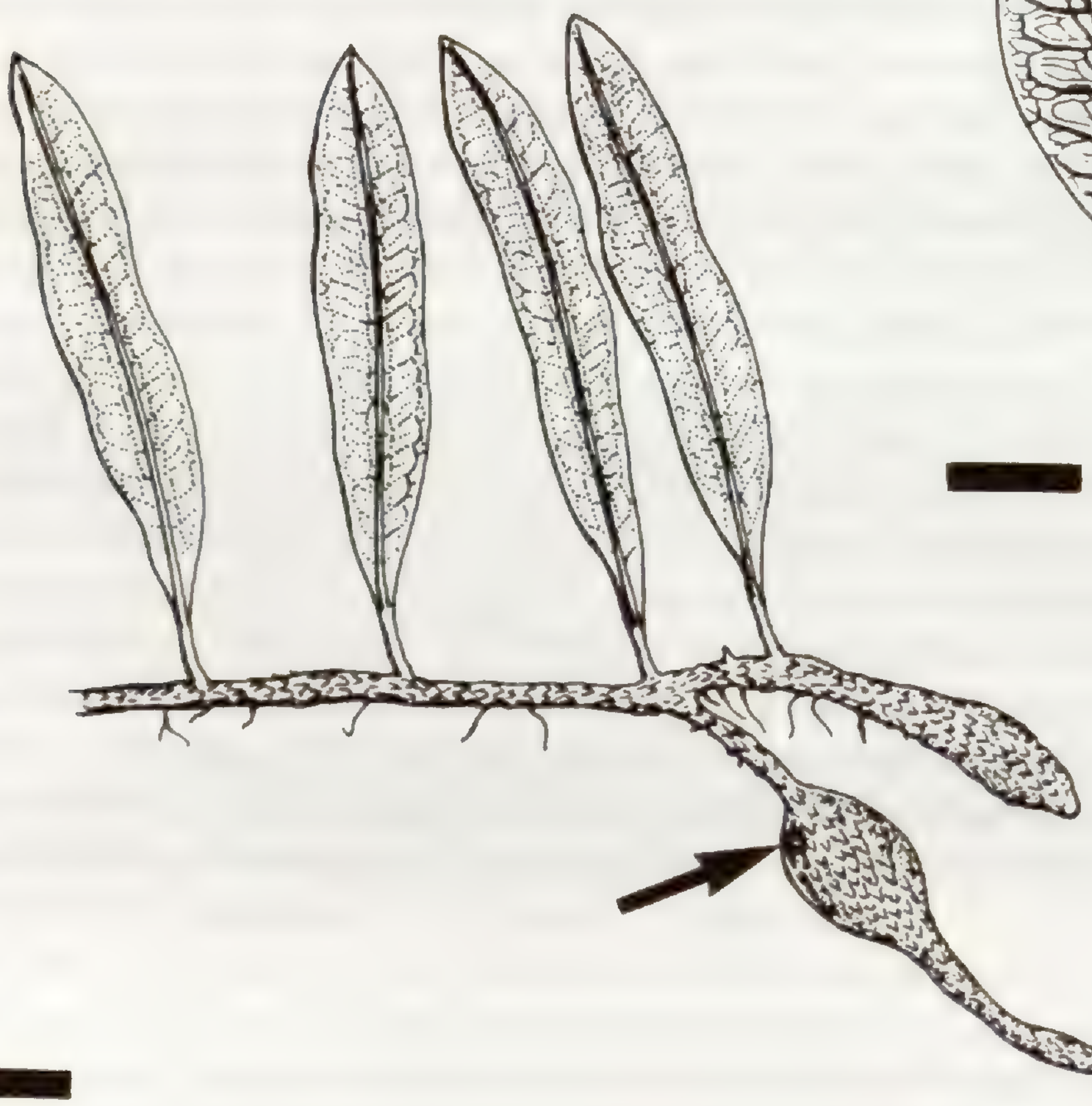
Results

Microgramma squamulosa (Polypodiaceae) is an ephiphytic fern with a creeping stem (Fig. 1), densely covered with scales; the leaves are spread in such a way that nodes and internodes can be distinguished. The leaves occur only on the dorsal side of the stem, while the adventitious roots appear only on the ventral side. This organization has some effect on the internal structure; the stele is dorsiventral in its structure, although in cross-section the stem is still circular. The organization of the leaf veins is characteristic (Fig. 2).

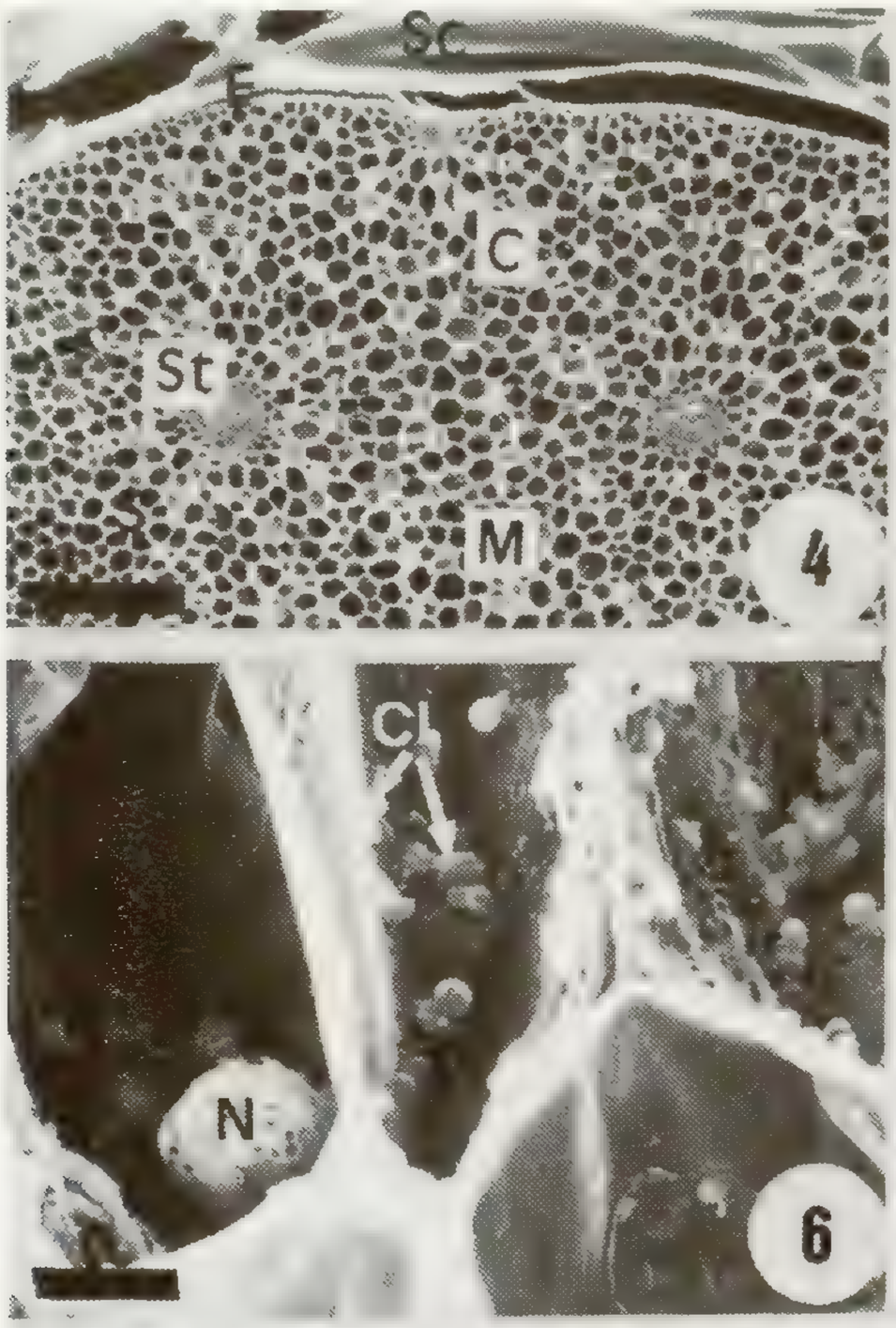
Stems of *M. squamulosa* with galls (Fig. 3) display peculiar characteristics. The affected region is fusiform in appearance. At least in external appearance, it no longer has the dordiventral arrangement of the unaffected regions. The dense covering of scales persists in the stem, but the leaves and the adventitious roots are generally lost. There appears to be no preferential region for the appearance of galls, they are found both on the main stem axis and on the branches, and occur on recent branches and on older stem regions.

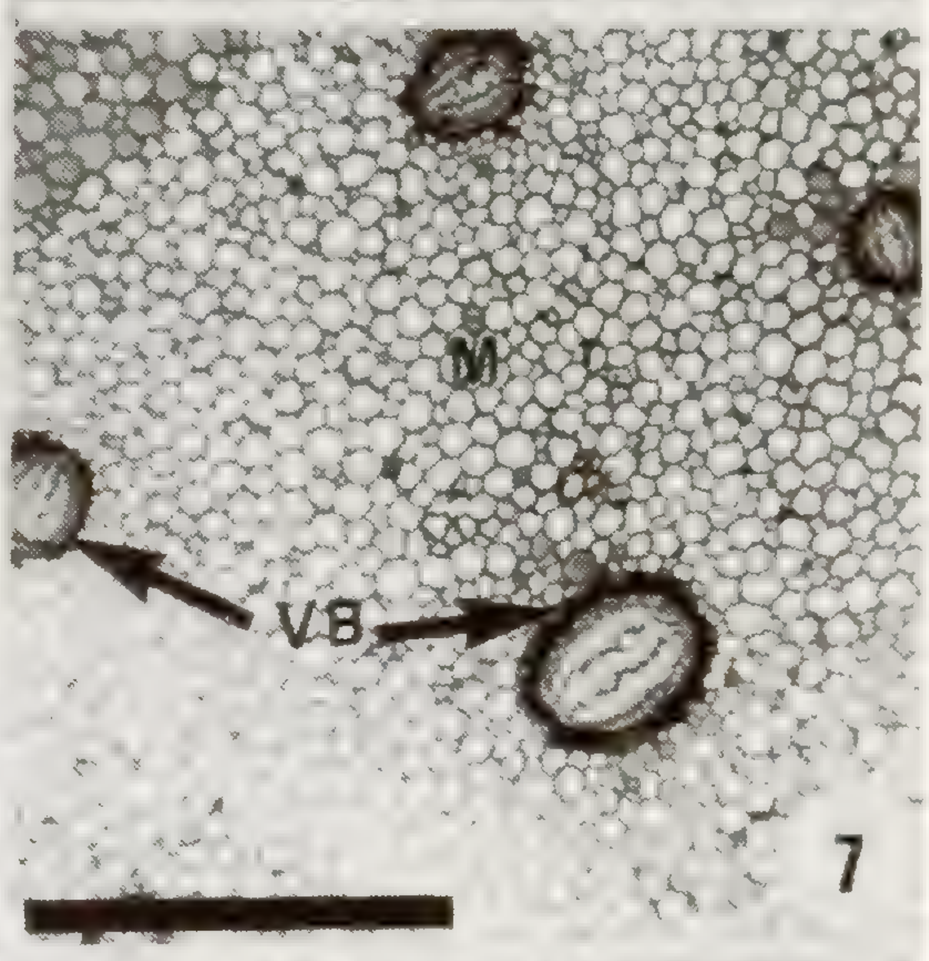
The gall inducer is a moth (Lepidoptera, Gelechiidae). At the more advanced stages of gall development a small orifice may be seen close to one of its extremities; this is previously prepared by the larva, and from it the inducing insect will emerge (Fig. 3).

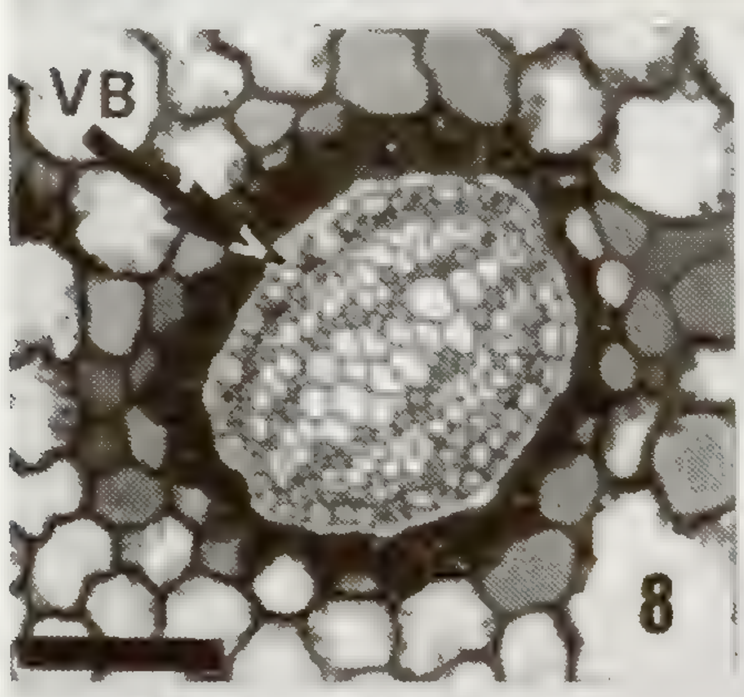
The internal structure of the unaffected stem on *M. squamulosa* is fairly simple. A cross-section shows, from the outside in, epidermis with scales, cortex, central cylinder or stele, and medulla (Fig. 4). The epidermis is composed of a single layer of quadrangular cells, and is covered by a fine cuticular layer and many scales (Fig. 5). The cortex and the medulla are made up basically of parenchymatic tissue, the cells of which are photosynthetic (Fig. 6). The stele of *M. squamulosa* is of the dictyostelic type – a cylindrical stele interrupted by numerous foliar lacunae (Fig. 7) –, so as to separate it into five or more meristeles. Each meristele (Fig. 8) is surrounded by the schlerenchyma sheath and the endodermis. The sclerenchyma (Fig. 9), or supporting tissue, is reduced; it is limited to a layer of quadrangular cells which surround the vascular bundles as a protective sheath. The cells display a brown-colored reinforcement in the tangential and radial walls. The endodermis (Fig. 9) may be distinguished by the regular cell arrangement; the cells are

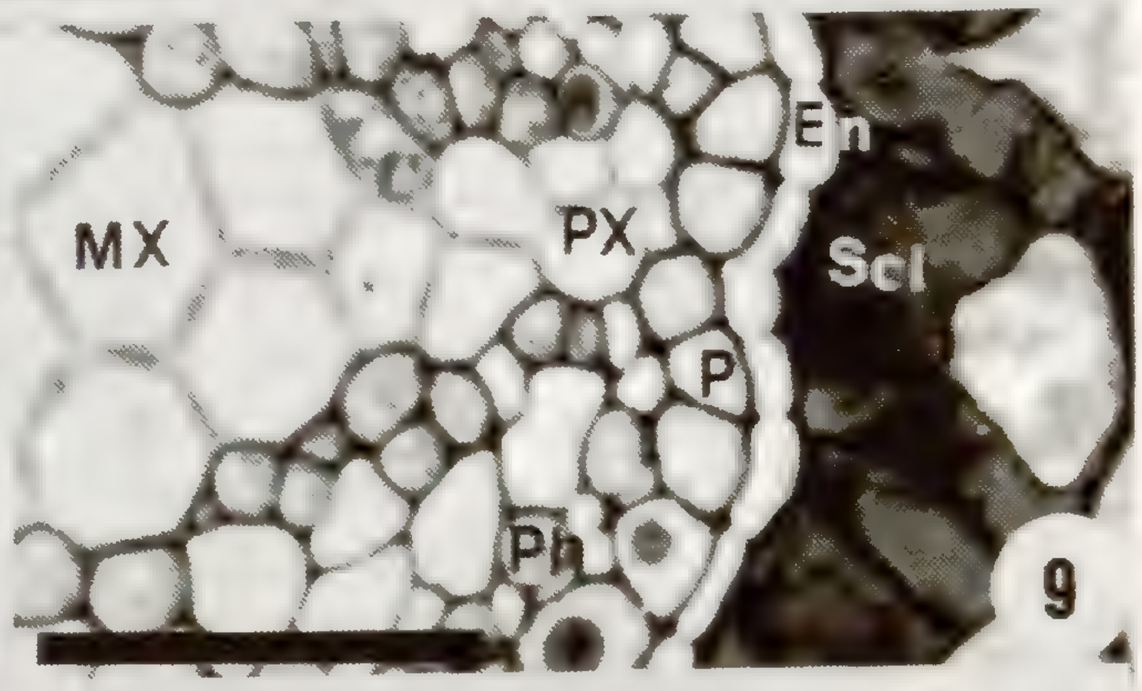


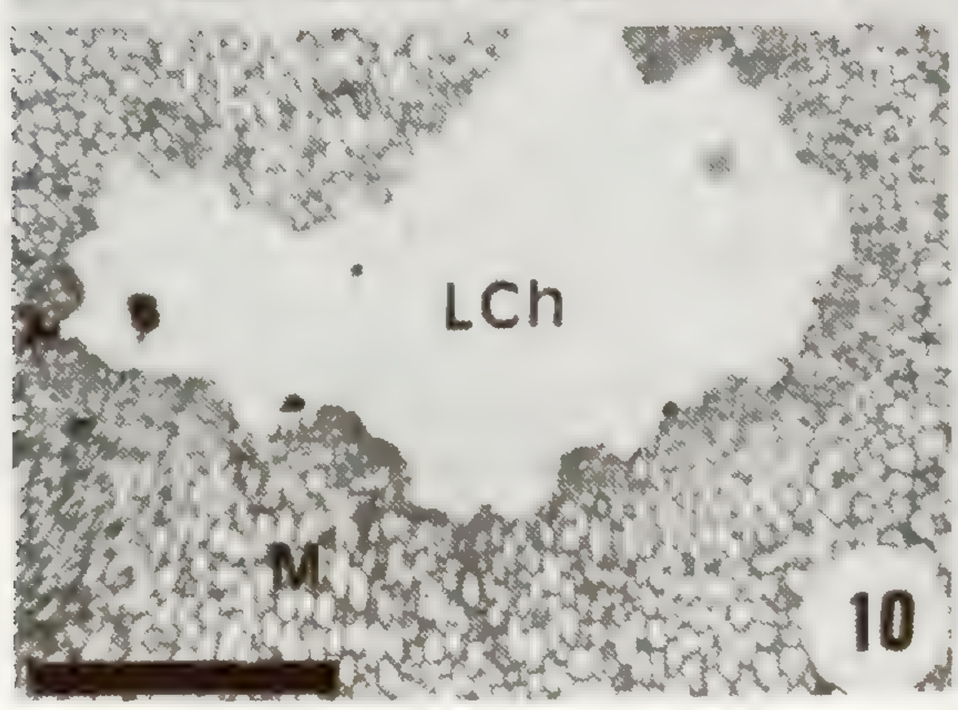
Figs. 1–3. *Microgramma squamulosa*. Fig. 1. General aspect of a plant without galls. Note the leaves and roots, respectively dorsal and ventral on the stems. Bar = 18 mm. Fig. 2. Note the characteristic vein pattern on the leaves. Bar = 10 mm. Fig. 3. General aspect of a plant with galls. Note the elongate fusiform shape of the stem gall. Where the gall occurs leaves and adventitious roots are generally lost. Observe the orifice by which the inductor leaves the gall (arrow). Bar = 18 mm.

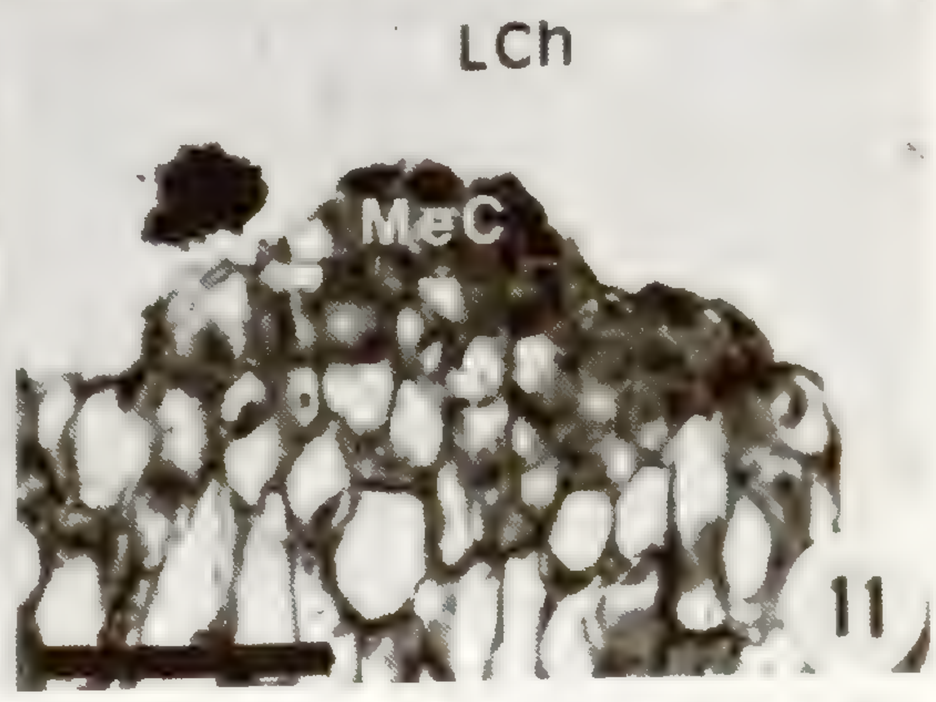












Figs. 4–11. *Microgramma squamulosa*. Figs. 4–9. Cross-section of a stem not affected. Figs. 9–10. Cross-section of young gall. Fig. 4. Note scales on the epidermis, cortex, stele and medulla (SEM). Bar = 300 µm. Fig. 5. Detail showing the scales on the epidermis. Bar = 500 µm. Fig. 6. Detail showing parenchymatic tissue of the cortex with nucleus and chloroplasts (SEM). Bar = 25 µm. Fig. 7. Note distribution of vascular bundles in the medulla. Bar = 1 mm. Fig. 8. General aspects of the vascular bundle. Bar = 22 µm. Fig. 9. Details of vascular bundle showing sclerenchyma, endodermis, pericycle, protoxylem, metaxylem, and phloem. Bar = 100 µm. Fig. 10. Note the larval chamber in the medullary region. Bar = 1 mm. Fig. 11. Detail of the meristematic cells of the larval chamber. Bar = 200 µm. C, cortex; Cl, chloroplast; E, epidermis; En, endodermis; LCh, larval chamber; M, medulla, MeC, meristematic cells; MX, metaxylem; N, nucleus; P, pericycle; Ph, phloem; PX, protoxylem; Sc, scale; Scl, sclerenchyma; St, stele; VB, vascular bundle.

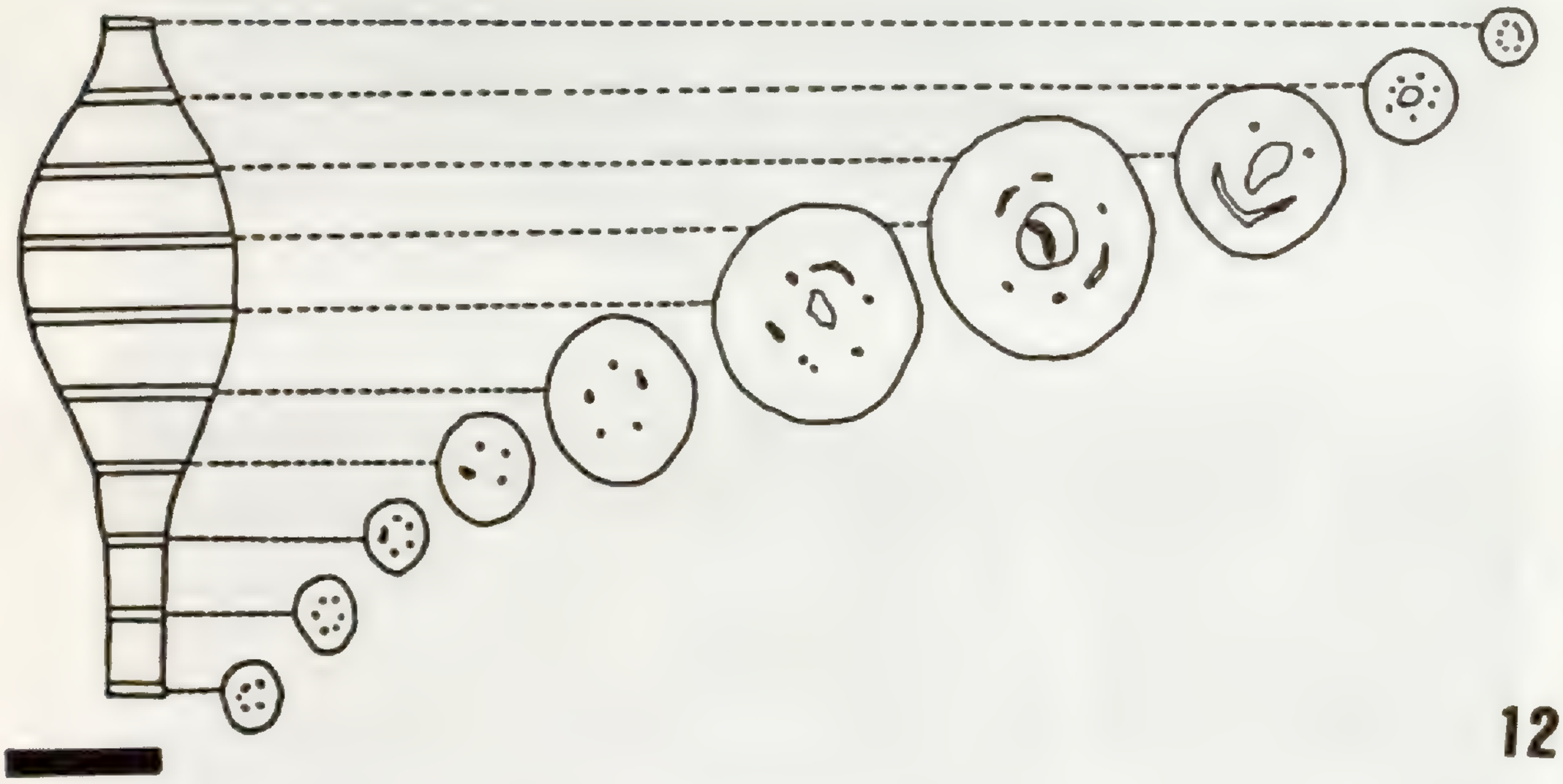


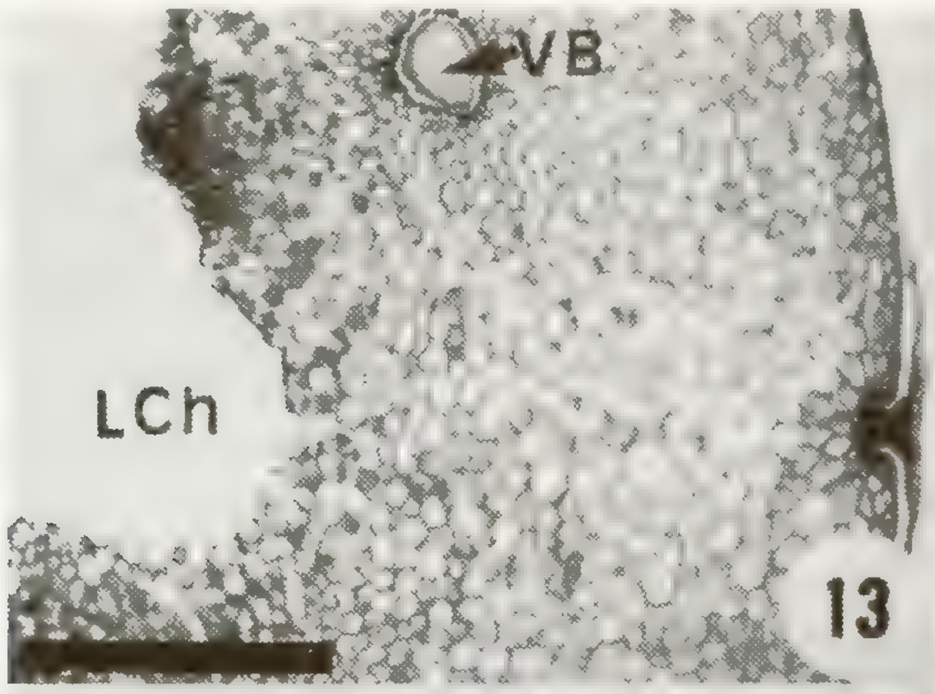
Fig. 12. *Microgramma squamulosa.* Diagram of cross-section series, showing bundle distribution in the gall. Bar = 10 mm.

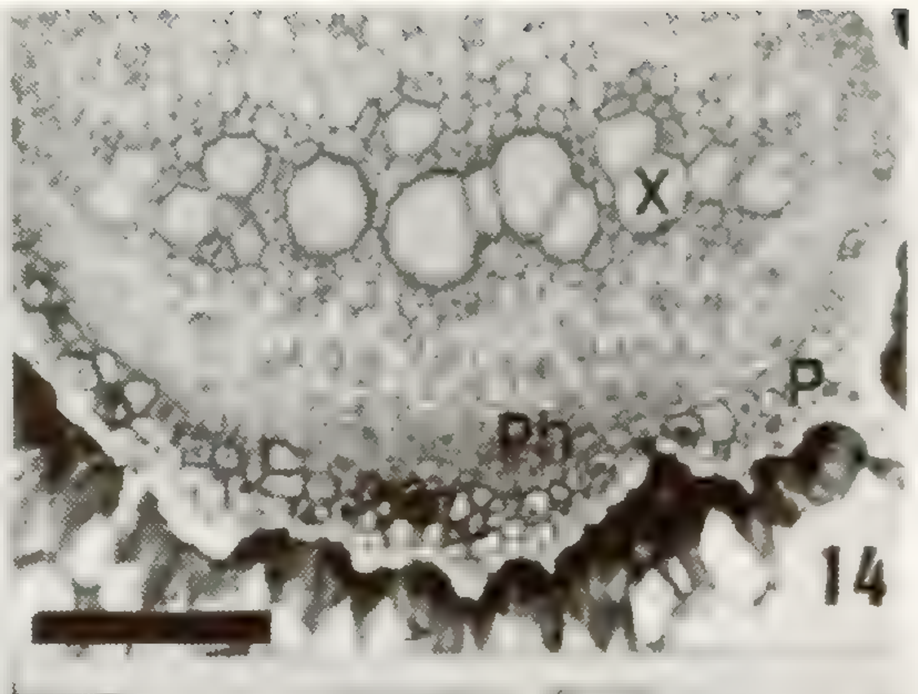
rectangular in shape, elongate in a tangential direction, and with thickening of the radial walls, characteristic of the presence of Casparian strips.

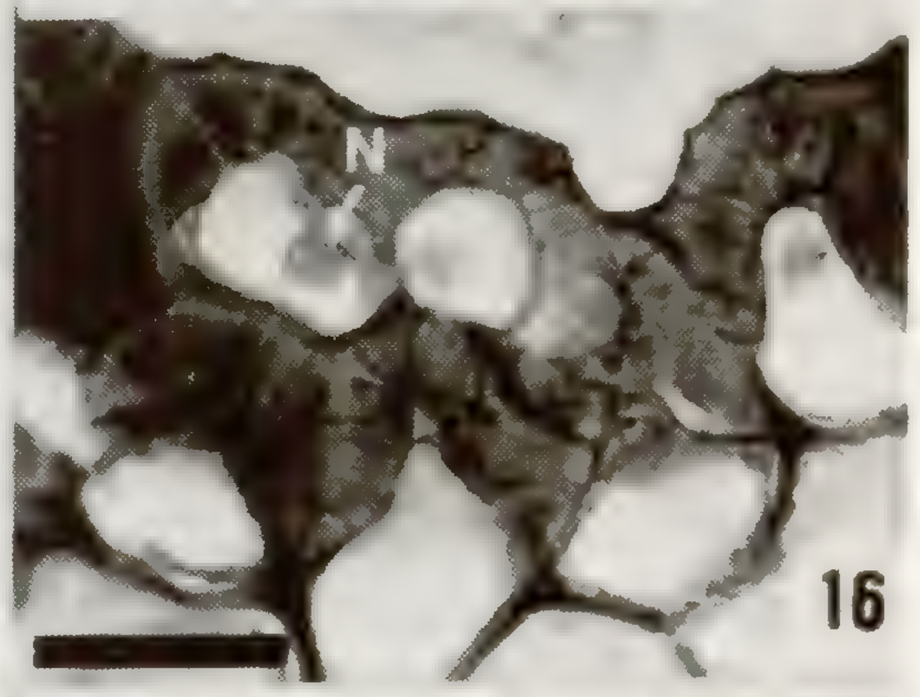
The entire vascular cylinder is concentric in organization; however, each meristele is formed by a bicolateral vascular bundle, with the phloem placed on both sides of the xylem (Fig. 8). The pericycle, situated between the phloem and endodermis, is generally made up of just one cell layer, quite distinct from both phloem and endodermis. Fig. 9 shows that, in association with the tracheal elements (tracheids) of the protoxylem and metaxylem, there occur the cells of the xylematic parenchyma, smaller and containing protoplasm. The conducting elements of the phloem (sieve cells) are quite simple in their pattern of differentiation: the protophloem remains at the periphery of each meristele, while the metaphloem differentiates centripetally. There is also the phloematic parenchyma. No significant difference has been observed between young and adult stems. Cross-sections of young galls show a relative small larval chamber formed in the medular parenchyma within the vascular cylinder (Fig. 10). The medullary cells which surround the larval chamber are meristematic in appearance – that is, the cells are smaller, contain dense cytoplasm, and have a conspicuous nucleus (Fig. 11). At this stage of gall development, no significant change is observed in the tissues of the vascular bundle.

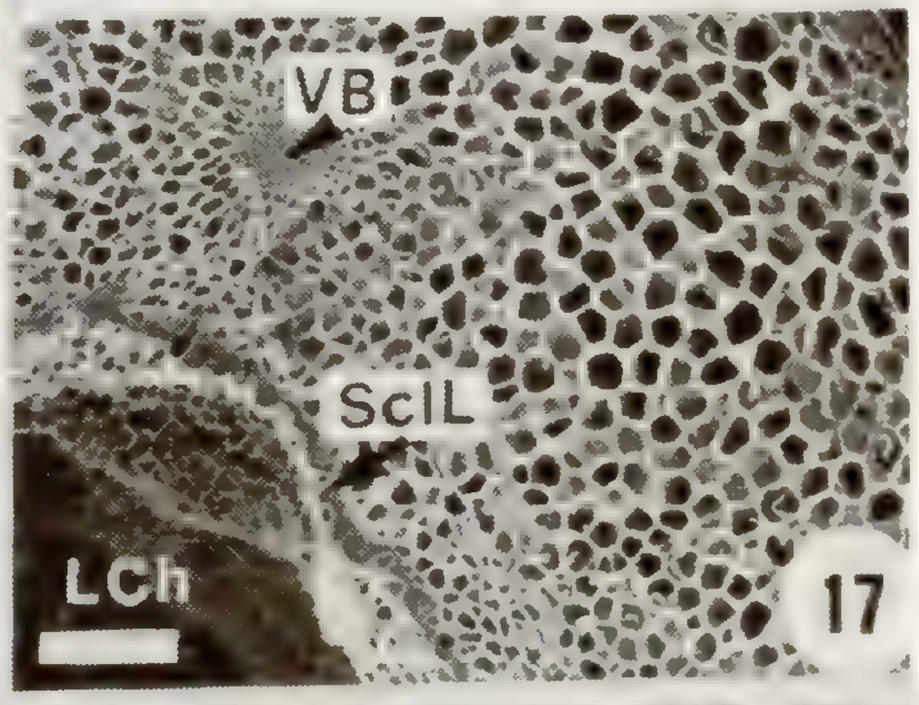
Cross-sections of stem segments at a more advanced stage of gall development (Fig. 12) show the occurrence of profound alteration in the distribution of the vascular bundles; these now display lateral expansions, forming bridges between neighboring meristeles. At this stage almost all the medullary region has been consumed by the larva, up to the immediate area of the vascular cylinder (Fig. 13). Profound alteration is also seen in the vascular bundle (Fig. 14 and 15) become larger, and their nucleus is also evident; the cytoplasm stains intensely with Safranin. Various layers of pericycle are formed as a result of cellular division.

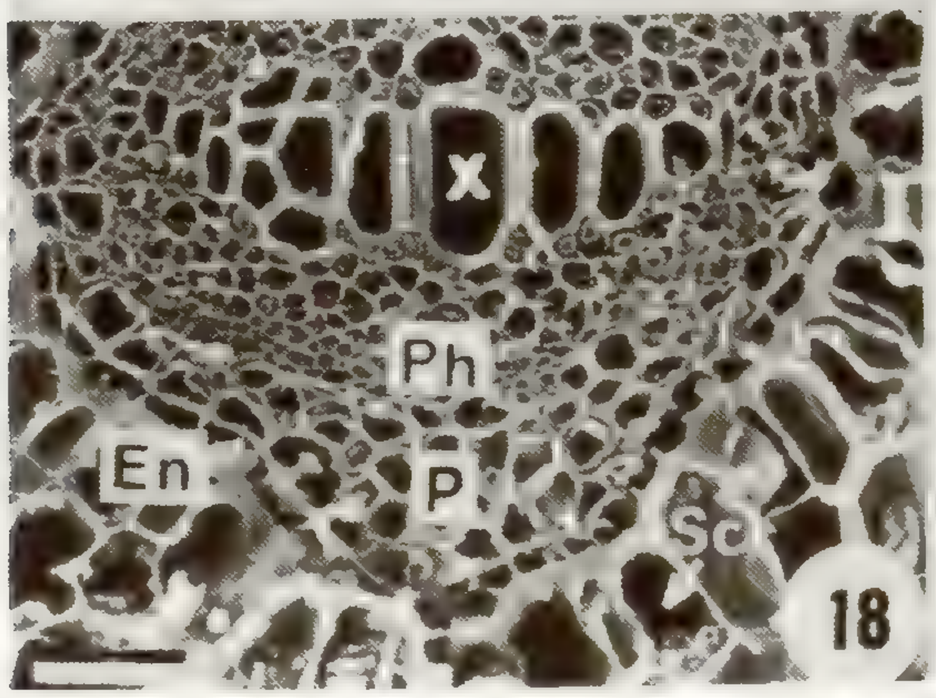
In cross-sections of the fully developed gall, the meristele has the same aspect (Fig. 16

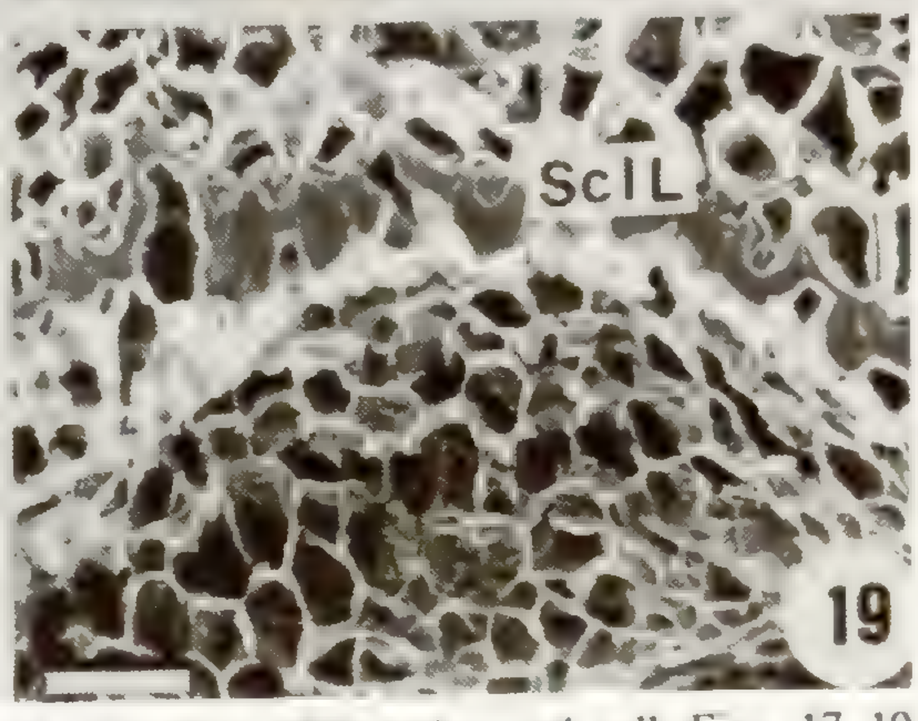












Figs. 13–19. *Microgramma squamulosa*. Figs. 13–16. Cross-section of the advanced gall. Figs. 17–19. Cross-section of fully developed gall. Fig. 13. Note the vascular bundle near the larval chamber. Bar = 1 mm. Fig. 14. Detail of the vascular bundle showing xylem, phloem and pericycle. Bar = 11 μm. Fig. 15. Details of part of the vascular bundle. Observe schlerenchyma, endodermis, cell layers of the pericycle, phloem and proto and metaxylem. Bar = 50 μm. Fig. 16. Note the nucleus of the living schlerenchyma cell. Bar = 25 μm. Fig. 17. Observe the larval chamber and the vascular bundle. Note the schlerenchyma-like tissue near the larval chamber (SEM). Bar = 300 μm. Fig. 18. Detail of the vascular bundle. Note cell layers of the pericycle, phloem and xylem (SEM). Bar = 30 μm. Fig. 19. Detail of the larval chamber. Note the layer of sclerenchyma-like cells around the larval chamber (SEM). Bar = 100 μm. En, endodermis; LCh, larval chamber; MX, metaxylem; N, nucleus; P, pericycle; Ph, phloem; PX, protoxylem; Scl, schlerenchyma; ScIL, schlerenchyma-like cells; VB, vascular bundle; X, xylem.

and 17), but in the region of the medula, surrounding the larval chamber, the cells no longer show meristematic characteristics; there occurs instead a layer of cells with thickened walls, similar to the sclerenchyma cells (Figs. 16 and 18).

Discussion

The insect which induces the gall of *Microgramma squamulosa* belongs to the family Gelechiidae. According to Borror & Delong (1969) the microlepidoptera in this family have larvae with a variety of habits: there are leaf miners, leaf rollers, leaf cutters, gall inducers, and pests of stored grain. The authors state that gelechiids of the genus *Gnorimoschema* cause stem galls in a variety of plants, particularly in the Asteraceae. These galls are elongate, fusiform, with rather thin walls. The galls caused by *M. squamulosa* are also elongate, fusiform, but the walls are not thin. According to Borror & Delong (1969) the larva, before entering pupal phase inside the gall, prepares an aperture in the upper part, and leaves it partly closed. When the adult insect hatches, it can get out easily. This is also the case with the gall inducer studied here.

According to Ogura (1972) the family Polypodiaceae is distributed worldwide, and the fossil record dates back to the Palaeozoic. More than 1,000 species are known; most have creeping stems, covered with scales, and trichomes of a variety of forms. The leaves are dispersed along the stems. *M. squamulosa* lacks trichomes, but otherwise conforms to this general description. In the region where the gall is formed the stems usually lack leaves and aventitious roots. This effect of gall formation has not been studied here; it may be due to the involvement of hormones. It is interesting to note that, in this case, gall development does not involve developmental processes which are abnormal to the plant; the relevant cells and tissues are abnormal only in that they occur in unusual quantities and places. The same observation has been made for angiosperms by Mani (1964) and by Rohfritsch & Shorthouse (1982). In the case of *M. squamulosa,* the relevant modified structures and processes are the formation of nutritive tissue, the cellular proliferation in the region of the pericycle, and the presence of sclerified tissue around the larval chamber.

Structural and metabolic modifications in the cells close to the larval chamber have already been described by Rohfritsch (1971), Bronner (1977), Rohfritsch & Shorthouse (1982), Meyer & Maresquelle (1983) and Shorthouse (1986) for other types of gall. The modifications cause the formation of nutritive tissue. The cells of the nutritive tissue characteristically display dense cytoplasm, fragmented vacuoles, prominent nuclei and nucleoli, and a large number of organelles (Bonner, 1977; Rohfritsch & Shorthouse, 1982; Shorthouse, 1986). These characteristics are, in general terms, observed in the galls of *M. squamulosa.* According to Rohfritsch & Shorthouse (1982) the lepidopteran larvae which cause galls feed by mastigation, as is the case with the inducing larva of *M. squamulosa,* that actively consume the tissue mass which surrounds the larval chamber. Here the galls are caused by the action of the larva itself, and not, as in many other cases, including galls caused by Hymenoptera, by secretions deposited at the moment of oviposition.

The influence of gall formation on the vascular system of the host, even at some distance from the larval chamber, has been commented by Meyer (1969), Meyer & Maresquelle (1983) and by Arduin *et al.* (1991). The fact may cast some light on the cytological and histological alterations in the pericycle: the increase in the number of cells layers, and in the size of the cells and their nuclei, and the intense colour of the cytoplasm when stained with Safranin. This last feature may be associated with a possible

increase in the amount of phenolic substances in The cells, thus favouring impregnation with the stain. Phenolic substances have already been detected in the Polypodiaceae by Ogura (1972), but in other tissues. According to this author, the brown colour of the walls of the sclerenchymatic cells is probably due to impregnation with phlobaphgene, which would have the effect of preventing weakening of the tissue. According to Feeny (1970, 1976) and Tempel (1981), one of the functions of phenolic substances is to defend the plant against herbivory. The phenolic substances in the pericycle cells may also act in defense of the tissue.

The presence of mechanical tissues, made up of cells with thickened and lignified walls, is widespread in protoplasmic galls. The cells of gall schlerenchyma derive in general from parenchymatic cells (Mani, 1964). Thus the formation of sclerified tissue around the larval chamber in a fully-developed gall of *M. squamulosa* is not an exceptional case, and such cells are derived from the medullary parenchyma.

Although the idea that gall formation acts in defense of the plant has met with little acceptance, the observation of *M. squamulosa* seems to favour the hypothesis. The formation of a pericycle with various cell layers around the larval chamber may be a physical barrier, to prevent alterations to the conducting elements, fundamental for the survival of the plants. Taft & Bissing (1988) state that in oak stem galls, development causes a blockage of the conducting elements of the functional xylem, and where attack is very severe, branches or even the entire plant may die. Sclerenchyma development around the larval chamber in galls of *M. squamulosa* may thus impede the advance of the inducing larva up to the conducting tissue. But it should also be kept in mind that the formation of sclerified tissue may also serve to protect the inducing larva against predators (Cornell, 1983).

More profound study of galls in pteridophytes would be enlightening, since the relative anatomical simplicity of the group makes it possible to work on a perturbation of this nature using just a few variables; this is a model material for better understanding of the intricate interactions between insect and plant. According to Ogura (1972), the most differentiated tissue system in the pteriodphytes in general is the stele, which thus represents an important feature for anatomical investigation.

ACKNOWLEDGMENTS

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Shorter Note

***Isoëtes reticulata* R. S. Hill 1987 (Alcheringa 12:158) is an illegitimate name.** — This name comes after *Isoëtes reticulata* C.B. Gena & T.N. Bhardwaja 1984. Three new species of genus Isoëtes L. from Rajasthan, India. (J. Bombay Natural History Society 81:165–168). The paper by Hill titled "Tertiary *Isoëtes* from Tasmania" (Alcheringa 12:157–162) is a very readable account about a Tasmanian fossil that is highly exciting to Isoetologists. There are four pictures of megaspores (Fig. 1, A–D) taken by SEM, showing striking reticulations of great clarity. These figures are compared with two from modern day species (Fig. 1, E–F). What is amazing is the fossil spores are from sediments containing leaf and root fragments that are not less than 22 million years old! Hill had sufficient quality material to assign it to the genus *Isoëtes* rather than the fossil genus *Isoëtites.* I am pleased to name this interesting fossil *Isoëtes* from Tasmania in honor of Dr. R.S. Hill.

***Isoëtes hillii* D. M. Britton, nom. nov. for *I. reticulata* Hill (1987), non Gena & Bhardwaja (1984).** — Donald M. Britton, Department of Molecular Biology and Genetics, University of Guelph, Guelph, Ontario, Canada, N1G 2W1.

AMERICAN FERN JOURNAL: VOLUME 83(4): 129-130 (1993)

New *Pellaea* from Brazil

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The following new species is described as a result of work on the cheilanthoid ferns (*Pteridaceae,* subfamily *Cheilanthoideae*) from the Espinhaço Range, Minas Gerais. This work was done as part of my Master's thesis at the University of São Paulo (Prado, 1989).

Pellaea cymbiformis J. Prado, **sp. nov.** Figure 1, A–G. *P. riedelii* Baker, cui affinis, frondibus monomorfis, erectis; petiolo ac rachidi sulcatis; folis lamina coriacea, 2–3 pinnato-imparipinnatis; pinnulis cymbiformibus; nervis immersis occultis differt. TYPE: Brazil, Minas Gerais: Gouveia, Km. 66 of the road from Curvelo-Diamantina, 9 April 1982, *Furlan* et al. s.n. *CFCR 3271* *Collection Flora of "Campos Rupestres". (Holotype SPF; isotypes K, HB, MBM).

Plants terrestrial. Rhizome short, ascending, ca 0.9 cm broad, bearing many scales; scales long-lanceolate, 0.3–0.9 cm long, reddish, concolorous. Fronds erect, monomorphic or nearly so, 5.5–17.0 cm long, borne in a cluster; stipe with 1 "U-shaped" vascular bundle at the middle, 2.0–9.5 cm long, ca. 0.1 cm diam., stipe and rachis sulcate adaxially, ruddy-tan to red becoming darker and gray with age, the distal portion of the rachis usually flexuous; lamina bi-tripinnate, imparipinnate, glabrous, coriaceous; segments ca 0.5 cm long and 0.1 cm broad, cymbiform, the margin revolute, border modified as indusium; veins free. Sporangia with short stalks; spores trilete, cristate.

Although the distinction of the two sections, *Pellaea* and *Ormopteris,* as presented by Tryon & Tryon (1982), is not clear, I believe that the new species described here, belongs to the *Ormopteris* section. *Pellaea cymbiformis* is closely related with other species *(P. pinnata* (Kaulf.) Prantl, *P. gleichenioides* (Hook.) Christ and *P. riedelii* Baker) previously included in this section (Tryon & Tryon, 1982) I think that a good character that distinguishes these two sections is the morphology of the indusium. In the section *Ormopteris* the indusium is abruptly and strongly differentiated from the margin while in the section *Pellaea* it is gradually and slightly to moderately differentiated from the margin.

Thus, *Pellaea cymbiformis* belongs to the section *Ormopteris,* a small section of 5–7 species of South America, all but one in Brazil (Tryon & Tryon, 1982). This placement is evinced by short stems with clustered stipes, rachises, and pinna-stalks strongly grooved on the adaxial side, indusium strongly differentiated from the margin, and cristate spores. Within the section *Ormopteris,* the species is caracterized by the cymbiform segments with diferenciated, revolute margins modified as indusia. The plants are usually about 11 cm long. This species resembles *Pellaea riedelii* in the 2–3-pinnate division of the lamina, but differs by the smaller, cymbiform segments. So far, this new species is known only from the type, which was collected in open, sunny places between rocks. It is probably endemic to the Espinhaço Range, Minas Gerais, Brazil.

I thank Dr. Paulo G. Windisch for assistance and suggestions.

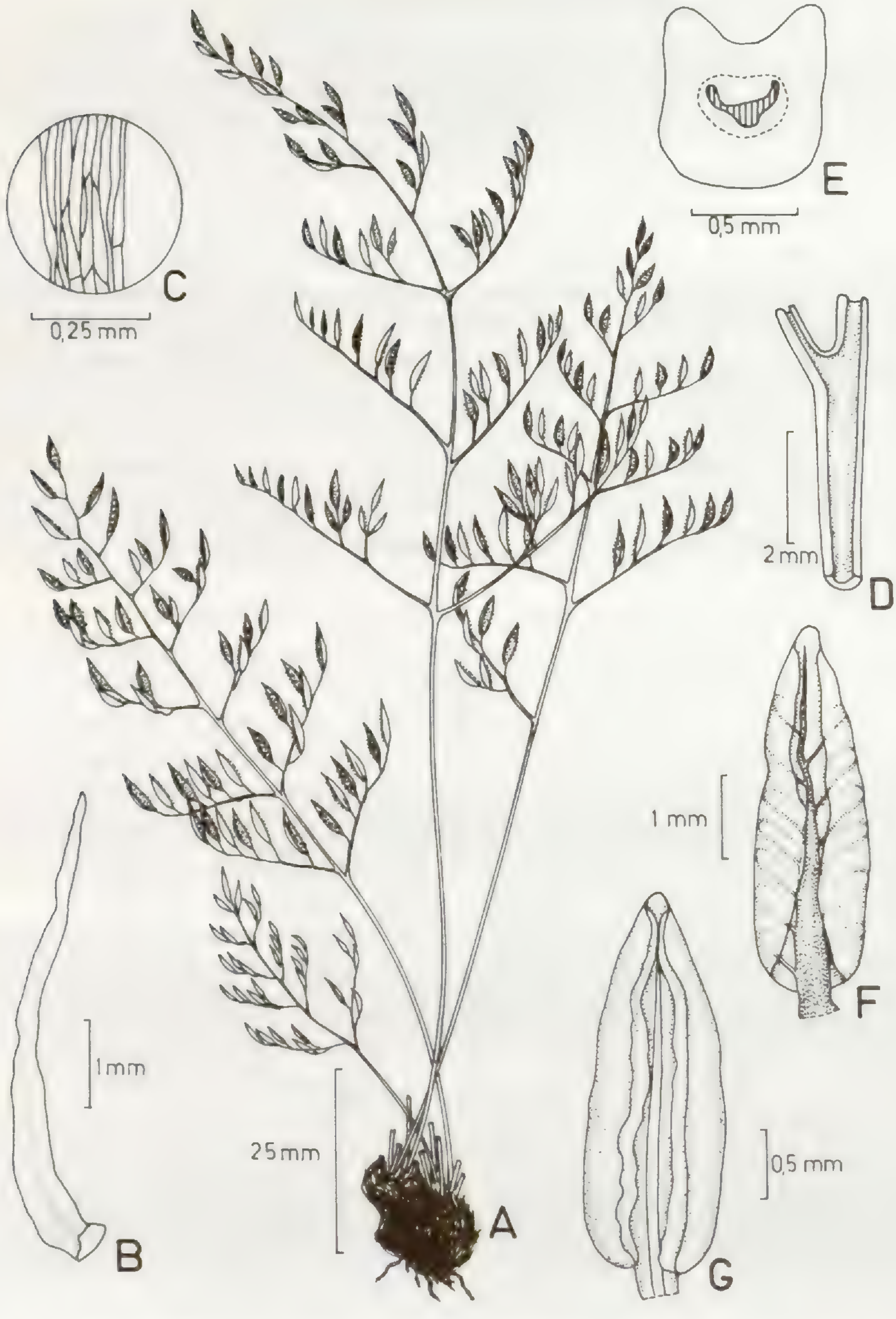

Fig. 1 A–G. *Pellaea cymbiformis* J. Prado. A, habit. B, rhizome scale. C, detail of the cells of a rhizome scale. D, stipe, E, transverse section of the stipe middle showing the vascular bundle. F, venation of sterile frond. G, venation of fertile frond.

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New Name and New Status in Brazilian *Pteris* L. *(Pteridaceae)*

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During my studies involving the genus *Pteris* from Brazil (Prado, 1993) I detected two taxa which need nomenclatural and taxonomic arrangement. Thus, one new name and one new status are presented here.

NEW NAME

Pteris gracilis was described by Fée (1852) based on a specimen collected by Claussen in Brazil (Rio de Janeiro). The epithet applied by Fée is illegitimate by homonymy, according Art. 64.1 of the Berlin Code (Greuter *et al.*, 1988). However, this species is really different, and its reocgnition is necessary. According to Art. 7.9 of the Code, a new name can be created, basedon the same type. Therefore, the new name proposed is:

Pteris congesta J. Prado **nom. nov.,** for *P. gracilis* Fée, 5 mém.: 128. 1852, with the same type.

BASIONYM: *Pteris gracilis* Fée, 5 mém.: 128. 1852. TYPE: Brasil, Rio de Janeiro, *Claussen* s.n. (*Isotype* RB!, photos K!, SPF!). nom. illeg. non Michx. (1803) nec Roxb. (1844).

Pteris congesta is characterized by 3-pinnate-pinnatifid ("congestus") frond with the short segments, pinnae and/or segments margin cleary denticulate-ciliate, venation free with the apex of the vein projecting as a cilium at the margin, and winged rhachis and racheola (Fig. 1A, B, C, D, E, F). It is closely related to *P. leptophylla* Sw. from which it differs mainly in the frond architecture (less pinnatifid) and venation partially aerolate, with large areoles close to the costa and costule, and venation free near by the margin (Fig. 1, G, H).

Both species are terrestrial plants, living from the sea level to 1000 m elevation, and endemic to Brazil. *Pteris congesta* is restricted to the State of Rio de Janeiro and *P. leptophylla* presents a wider range of ocurrence, from the State of Pernambuco to Santa Catarina.

NEW STATUS

Raddi (1819) described *Pteris tristicula* Raddi, based on specimens collected from Rio de Janeiro, Brazil. Raddi mentioned that this species presented "pinnis pinnatifidis et rachis alata." During the course of a taxonomic treatment of *Pteris* from Brazil, I analyzed many collections, from different places, including material from Argentina and Paraguay, and it was possible to recognize that Raddi's taxon represents a variety of *P. denticulata* Sw., and this new status is proposed herein:

Pteris denticulata Swartz var. ***tristicula*** (Raddi) J. Prado **stat. nov.** for *Pteris tristicula* Raddi, Opusc. Sci. Bol. 3:293. 1819.

Pteris tristicula Raddi, Opusc. Sci. Bol. 3: 293. 1819. TYPE: Brasil, Rio de Janeiro, Catumbi, *Raddi* s.n. (*Holotype* FI!, photos BM!, K!, SPF!; *isotype* BR!, photos BM!, K!, SPF!).

Litobrochia tristicula (Raddi) Presl, Tent. Pter. 149. 1836.

Pteris denticulata var. *tristicula* is characterized by winged rhachis, margin or pinnae

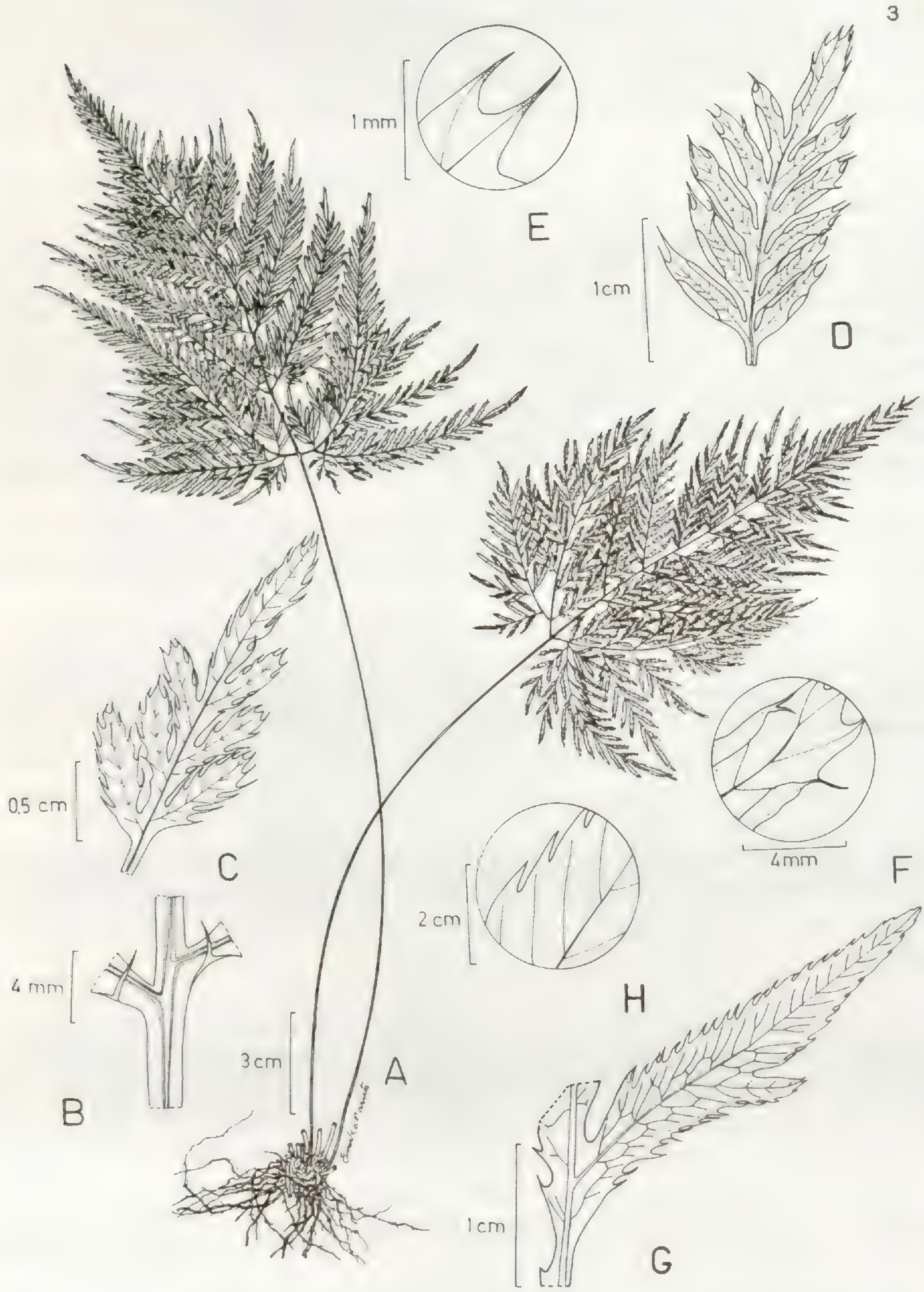

Fig. 1. A–F, *Pteris congesta* J. Prado. A, habit. B, winged rhachis. C–D, sterile and fertile pinnae, respectively. E–F, details of pinnae (sterile and fertile, respectively) showing the denticulate-ciliate margins and the free venation. G–H, *P. leptophylla* Sw. G, sterile segment, showing the partially areolate venation. H, detail of the segment margin denticulate.

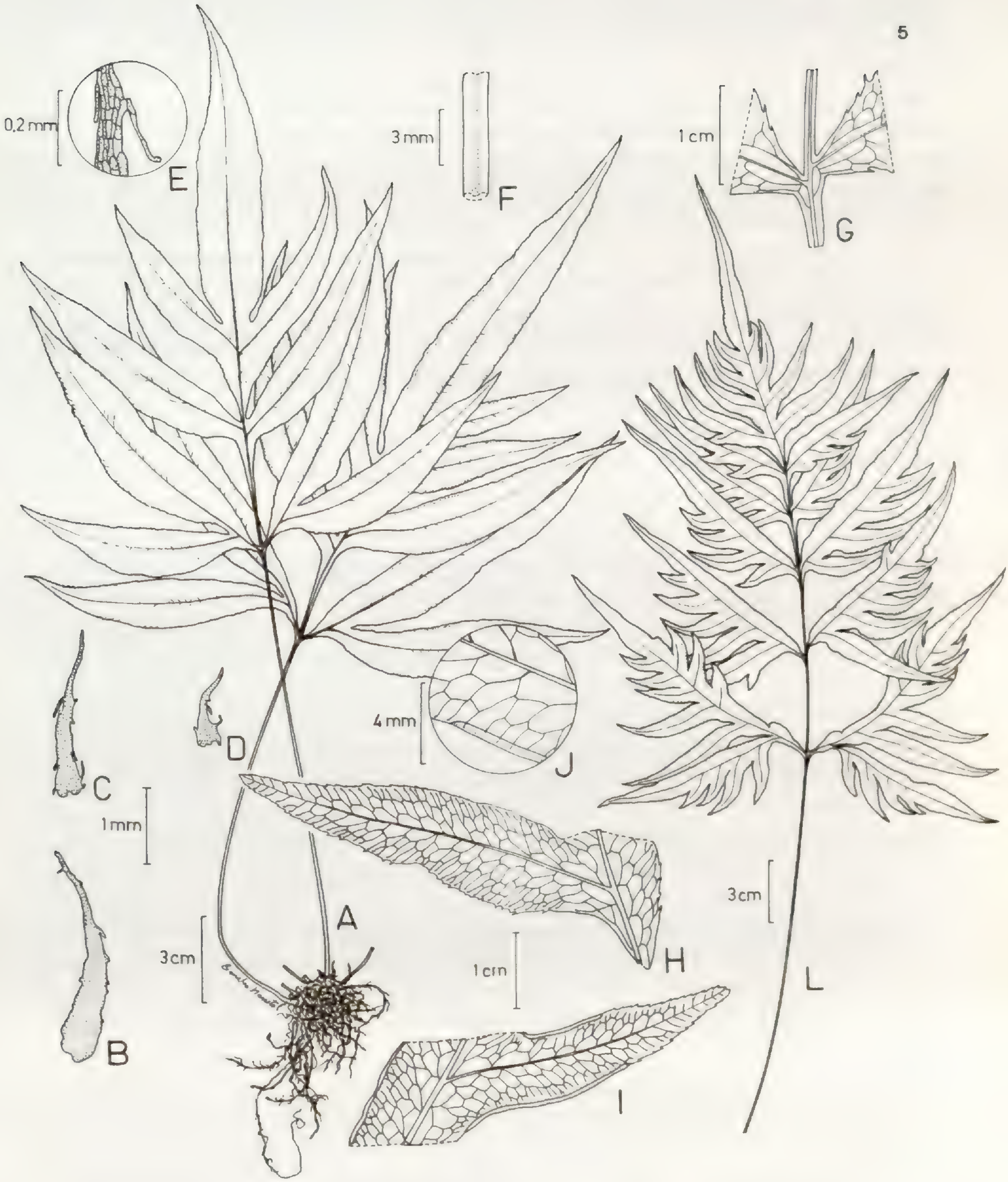

Fig. 2. A–J, *Pteris denticulata* Sw. var. *denticulata*. A, habit. B, C, D, rhizome scales. E, detail of the cells of a rhizome scale. F, stipe. G, winged rhachis. H–I, venation of sterile and fertile fronds, respectively. J, detail of the frond margin. L, var. *tristicula* (Raddi) J. Prado, habit.

denticulate, and basal, median, apical pinnae pinnatifid, mainly at basioscopic side (Fig. 2, G, H, I, L). The last character differ it from the variety *denticulata*.

The variety *tristicula* is represnted by terrestrial or rupiculous plants growing in forest of island and mountainous regions of Brazil and Argentina, from the sea level to ca. 1000 m elevation. It is widely distributed in Brazil, from the State of Ceará to Santa Catarina.

ACKNOWLEDGMENTS

The author wishes to thank Dr. Paulo G. Windisch for his guidance and useful suggestions, the curators of the following herbaria FI, BM, BR, RB for providing the botanical material, and Emiko Naruto for the illustrations.

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Review

Scandinavian Ferns by Benjamin Øllgaard and Kirsten Tind. 1993. Rhodos, International Science and Art Publisher, Copenhagen. 317 pp, 103 line drawings, 114 color plates. Folio (25 x 34 cm). Paperback, ISBN 87-7245-530-6, Dan. Kr. 375.00; Hardback, ISBN 87-7245-532-2, Dan. Kr. 425.00.

The 72 species of pteridophytes in the Scandanavian region (Denmark, Norway, and Sweden) are amply described and discussed as to their systematics, morphology, ecology, distribution, and economic uses. The keys are simple and quite workable. The technical aspects provide a most useful floristic tool, even though the large size renders field-use unlikely. There is a good reason for making this book suitable for the coffee-table. The full-color illustrations by K. Tind are visually stunning, reminiscent of botanical artwork of generations past. The artwork alone provides a splendor sufficient to sustain one throughout a long winter. Many of the illustrations show the habit and associates of the ferns, particularly useful in knowing and locating the more diminuative members of the flora. The authors are to be congratulated. — JAMES H. PECK, Department of Biology, University of Arkansas at Little Rock, Little Rock, AR 72204.

Shorter Note

Vegetative Reproduction Common in *Ophiglossum pycnostichum*. — The common southern adder's tongue, *Ophioglossum pycnostichum* (Fern.) Love & Love, is widely distributed throughout the moist woods of Southeastern United States. It is probably the most commonly noticed species of *Ophioglossum* because of its relatively large size (to 30 cm tall in my specimens) and so has been widely collected. *O. petiolatum* Hooker may reach a height of 20 cm or more but is uncommon. Despite the large number of *O. pycnostichum* specimens that have been collected, its growth habit is most often described as "plants growing singly" occasionally acompanied by a note such as "rarely spreading by means of proliferous buds on the roots." Though vegetative reproduction is rather common in *O. petiolatum, O. nudicaule L.* and *O. englemanii* Prantl, until now, we have only seen *O. pycnostichum* growing singly.

Here we report two separate and distinct Louisiana populations in which *O. pycnostichum* has been collected reproducing vegetatively by means of proliferous buds on the roots. In the first population, a specimen was collected that had two fully developed plants sharing a common root. On further investigation, these "doubles" were found to be quite common as well as "triples" (one plant that has vegetatively produced two distinct new plants). The most extraordinary find was a pair of large plants connected together that had given rise to eight additional plants between them (one individual made up a total of ten well developed plants). This population is approximately twelve meters long and three meters wide and is very densely populated. It is located in a semi-disturbed pine/hardwood forest on the east side of Bayou DeSiard ca 0.5 mile northeast of the intersection of US 165 and LA 134 north of Monroe, LA, 26 April 1993, *Cascio 126,* (NLU), 3 April, 1993, *Cascio 128–129* (NLU). The plants are growing in an area that is slightly depressed and remains moist longer than the area surrounding it.

A second population, approximately two miles from the first was then sampled. It was found to contain two "doubles" of only four plants examined. The plants in this population were few and scattered over an area approximately seven meters long and five meters wide. This population is located south of Keystone Road East of Sterlington, LA ca 1.7 miles west of the intersection of US 165 and LA 134, 4 April 1993, *Cascio 130,* (NLU). This is a mixed pine/hardwood forest prone to partial flooding.

The possibility of having observed a unique reproduction mechanism with so little effort and at such a rate seems unlikely. It is possible that the rarity of these types of collections is due more to collection method than rate of occurrence; however, specimens from more than these two populations would have to be more carefully examined for this habit before reaching such a conclusion. — David Keith Cascio and R. Dale Thomas, Department of Biology, Northeast Louisiana University, Monroe, LA 71209.

Referees for 1993

I thank the Associate Editors and the referees listed below for their assistance in the review process. Their evaluations of manuscripts submitted to the American Fern Journal have aided the authors, made by job easier, and contributed to the quality of our journal – James H. Peck.

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American Fern Journal

Volume 84

Number 1

January-March 1994

QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

Trichomanes polypodioides **and Its Allies**
David B. Lellinger **1**

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AMERICAN FERN JOURNAL 84(1):1–4 (1994)

Trichomanes polypodioides and Its Allies

DAVID B. LELLINGER
Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington, DC20560

The identity of neotropical specimens of *Trichomanes* is often puzzling because the species vary in frond size and dissection according to the humidity and light intensity under which they grow and because many of the species are wide ranging and have accumulated different names in different countries. Although there has never been a modern monograph of the genus as a whole, Wessels-Boer (1962) treated the species of subg. *Didymoglossum* (Desv.) C. Chr. and more recently Windisch (1993) published on the *T. crispum* group, which are among the most vexing species of subg. *Achomanes* K. Presl.

Trichomanes polypodioides L. is a rather common, widespread, lowland and mid-elevation epiphyte. It is often found on tree fern trunks and has short, strongly alate stipes and narrowly lanceolate, merely pinnatifid laminae with entire to sinuous or crenate lobes, a frond form that is rather unusual in *Trichomanes*. This species and its allies form section *Acarpacrium* (Prantl) C. Chr. of subg. *Achomanes* and are characterized by having stellate (or sometimes bifid) hairs, rather than simple hairs, on the lamina margins and usually on the veins. Morton (1968, pp. 198-199) included ten names in this section, five of which appear to be valid species. The disposition of the other names is as follows: *Trichomanes adscendens* Kunze is probably a synonym of *T. holopterum* Kunze, *T. attenuatum* Hook. and *T. ptilodes* Bosch are synonyms of *T. alatum* Swartz, *T. curranii* Weath. is a synonym of *T. superbum* Bosch, and *T. fimbriatum* Backh. is a synonym of *T. trigonum* Desv.

In studying these species for the Flora of the Guianas, I found some specimens that had been misnamed and were actually a new species:

Trichomanes caliginum Lellinger, sp. nov. **Figs. 1–3**

Rhizoma repens, 1–1.5(2) mm in diam. Frondes subdistantae monomorphicae; axibus venisque abaxialiter stellato-pilosis, maturitate saepe glabriusculis. Stipites (1.5)4–12(20) cm longa, 1–1.5(2) mm lata, late alata, alis aliquando decrescentibus, margine stellato-pilosis, radiis 2–4-cellularibus manifeste septatis. Rachides uniformiter late alatae, marginibus stellato-pilosis, radiis 1(2)-cellularibus. Laminae 6–21(27) cm longae, 3–8(14) cm latae, juventute oblongae bipinnatifidae, maturitate lanceatae bipinnato-pinnatifidae; pinnis (10)15–25(30) paribus supoppositis vel alternatis, juventute leviter lobatis, maturitate pinnatifidis, segmentibus distanter angusteque serrato-lobatis, marginibus stellato-pilosis, radiis 1(2)-cellularibus. Involucra immersa, 1–1.5 mm longa, ca. 0.75 mm lata, juventute brevicylindrica truncata, maturitate eroso-subconica, protuberationibus lateralibus ulterius apicibus involucrorum extensis.

TYPE: Venezuela: Edo. Bolívar: talus forest of Sarvén-tepuí, *Wurdack 34094* (US; isotype NY).

DISTRIBUTION: Southern Venezuela to Surinam. Terrestrial, often among mosses and boulders, or epipetric at (400) 800–2000 m elevation.

PARATYPES: **VENEZUELA:** Edo. Bolívar: Cerro La Danta, NW of Cerro Venamo, 1050 m, *Steyermark & Nilsson 28* (US); NW slopes of Cerro Venamo, 1200 m, *Steyermark & Nilsson 145* (US); Terr. Fed. Amazonas: Below Salto Los Monos on a trib-

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Figs. 1–3. Holotype of *Trichomanes caliginum* Lellinger (*Wurdack 34094*, US). Fig. 1. Plant. Fig. 2. Fertile pinna. Fig. 3. Detail of pinna lobes showing marginal hairs.

utary of the headwaters of the Río Iguapo, 3°35'N, 65°23'W, 1500–1650 m, *Liesner 18579* (US); Cerro Marahuaca, *Maguire 29178* (US). **GUYANA:** Ca. 2 km NW of the N tip of the prow of Mt. Roraima, 1000–1500 m, *Hahn 5446* (B, U, US); E slopes of Mt. Eboropu, 3–5 mi upstream on Saydak Creek from its junction with the Kako River, 470–1030 m, *Edwards 1139* (BRG, K, U, US); NW-facing slopes of Mt. Roraima near the end of the Waruma Trail, ca. 4200–4300 ft.; *Kew Exped. Roraima 19* (BRG, K, U, US); without definite locality, *Appun?* (Morton photo 18432, with apparently falsified data "[Brazil], Glaziou 12387"). **SURINAM:** Tafelberg, Arrowhead Basin, *Maguire 24620* (NY, P, US): Mt. Emmaketen, *Daniëls & Jonker 1237* (U, US).

This species has usually been misidentified as *T. curranii* Weath. or *T. trigonum* Desv., from which it differs by the characters given in the following key. The epithet *caliginum* means "of the clouds," in reference to its presence in cloud forests, as well as montane rain forests. Wurdack (1970) has published on erroneous data involving Glaziou collections; Alan Smith suggested that the unlocalized Guayana specimen probably was collected by Appun. The new species and its relatives can be distinguished by the following key:

1. Rhizomes ca. 0.5 mm in diam.
   2. Laminae lobed to deeply pinnatifid, the segments patent, obtuse; involucres terminal on the usually sinuous or crenate segments. Epiphytic; 0–2000 m elev. (ANTILLES, TRINIDAD, CENT. AMER., trop. SO. AMER.).
      T. polypodioides L.
   2. Laminae deeply pinnatifid, the segments subacuminate; involucres terminal on lateral lobules of the ascending, obliquely lobed segments (JAMAICA).
      T. pinnatifidum Bosch

1. Rhizomes 1–2 mm in diam.
   3. Proximal pinnae more distant than the distal ones and usually much shorter, the laminae often nearly elliptic; fronds usually less than 15 cm long. Epiphytic or terrestrial at high elevations; 400–2000 m. elev. (ANTILLES; coastal VENEZUELA).
      T. alatum Desv.
   3. Proximal pinnae not more distant than the distal ones and often much longer, the laminae lanceate to lanceolate; fronds usually more than 15 cm long (except in *T. alatum).*
      4. Laminae narrowly lanceolate, 4–6 times longer than wide, usually pinnatifid with oblong segments; cells of the laminae large; stipes narrowly alate. Terrestrial or fallen epiphytes; 300–1100(1400) m. elev. (LESS. ANTILLES, TRINIDAD).
         T. trigonum Desv.
      4. Laminae lanceate, 2–4 times longer than wide; cells of the laminae small; stipes broadly alate.
         5. Stipes longer than the laminae. Basal pinnae (except in juveniles) elliptic-lanceolate, 2–3 cm wide near the middle, the lateral segments mostly lanceate, ca. 8 pairs. Terrestrial or a low trunk epiphyte; 300–1300 m. elev. (TRINIDAD; coastal VENEZUELA).
            T. superbum Bosch

5. Stipes shorter than the laminae. Basal pinnae lanceolate, 0.5–1.5(2) cm wide near the middle, the lateral segments nearly oblong, 8–15 pairs. Epiphytic; 800–2000 m. elev. (so. VENEZUELA to SURINAM).
T. caliginum Lellinger

Trichomanes pinnatifidum is known only from the Jamaican type and to me only from the description by Proctor (1985, p. 124), who noted that the species may have been extirpated. *Trichomanes trigonum* var. *fimbriatum* (Backh.) Proctor (type specimen from cultivation) appears to be an ecological form induced by superhumid conditions that cause the lobes of the pinnae to elongate into an acuminate process one cell wide at the sometimes curled apex. This could be tested by growing var. *trigonum* under superhumid conditions or var. *fimbriatum* under relatively dry conditions. I have seen only two sheets of the latter, (Grenada, *Miller, Sherring,* both US). Despite its striking appearance, this form probably is not worthy of taxonomic recognition. Synonymies for *T. trigonum* and *T. alatum* can be found in Proctor (1977, pp. 92, 94) and for *T. polypodioides* in Lellinger (1989, p. 217), and so are not repeated here.

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Shorter Note

Trichomanes rigidum* versus *Trichomanes cupressoides. — The species of *Trichomanes* subg. *Pachychaetum* are unlike those of the other subgenera of *Trichomanes* in having highly dissected laminae borne on erect rhizomes that are usually terrestrial or epipetric, rather than epiphytic. The species of this subgenus number only about 25, of which six are present in the New World. A few of the species are wide-ranging and similar in many characters, and so have been confused. This is the case with *Trichomanes rigidum* Swartz (a common species of the lowland neotropics) and *T. cupressoides* Desv. (a common species of the lowland tropics in Asia and Africa). The late E. A. C. L. E. Schelpe (Fl. Zambesiaca 78. 1970) thought these were a single species. He also included *T. mandiocanum* Raddi, which is based on a Brazilian type, and *T. dregei* Bosch, which is based on a South African type, under Swartz' name. The following will distinguish the New World species from the Old World one: *T. rigidum:* Stipes adaxially terete, usually exalate; costae of proximal pinnae exalate; *T. cupressoides:* Stipes adaxially flattened to deeply sulcate, usually mostly alate; costae of proximal pinnae alate and often slightly surcurrent and decurrent on the rachis. — David B. Lellinger, U. S. National Herbarium MRC-166, Smithsonian Institution, Washington, DC 20560.

Another Widely Disjunct, Rare and Local North American Moonwort (Ophioglossaceae: *Botrychium* subg. *Botrychium*)

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Studies of North American moonworts (*Botrychium* subg. *Botrychium*) during the past two decades show that there are some 24 species, described and undescribed, instead of the traditional interpretation of only six (e.g. Clausen, 1938). Four times as many species occur in North America as in the rest of the world. In addition, there are many varieties and forms. North America is clearly the world's center of diversity of moonworts (Cf. Wagner & Wagner, 1981, 1983, 1986, 1990a, 1990b).

Many of the taxa are rare or very rare, some known from only one specimen from each of several localities, and many recognized only in the last two decades. Some evidently have very narrow total ranges such as *B. acuminatum* W. H. Wagner, *B. gallicomontanum* Farrar & Johnson-Groh, *B. mormo* W. H. Wagner, and *B. pumicola* Coville. At the other extreme are *B. lunaria* (L.) Sw., *B. lanceolatum* (Gmel.) Angstr., *B. minganense* Victorin, and *B. simplex* E. Hitchc., which are relatively common species. Of special interest are those species which have very large ranges but are sporadic with their populations being usually small and widely separated.

Among these is *Botrychium pallidum* W. H. Wagner, which is sporadic with small colonies of few individuals in Colorado, Saskatchewan, Michigan, Ontario, and Quebec. Another is the remarkable two-spike moonwort *B. paradoxum* W. H. Wagner, known from British Columbia, Alberta, Saskatchewan, Montana, Oregon, and Utah, with populations often made up of only one or several individuals. From the standpoint of comparative rarity, mere listings of provinces and states as done here can be misleading. If the actual populations are very small and sporadic, these taxa must be ranked as very rare and locally threatened or endangered. The situation is similar to that in the seedplants *Plantago cordata* Lam. in much of its range (except for the eastern Ozarks), and the dwarf whorled pogonia, *Isotria medeoloides* (Pursh) Raf. For such widely scattered populations, it is interesting to speculate on their origin and how the species maintain themselves.

The largest two colonies of the species described below comprise ca. 45 and 15 individuals, but the others are much smaller, some with only one or two plants, at least as judged from our available data. The species is so distinct that if it were more common, one would expect it to be readily recognized by collectors as different, and therefore sampled. Its distinctive characters are herewith enumerated:

Botrychium lineare W. H. Wagner, sp. nov.

Caulis usque ad 3.0 cm longus. Folium usque ad 18 cm longum. Tropophorum subsessile vel stipitatum stipite usque ad 1 cm longo. Lamina oblonga, semel pinnata. Pinnarum paria 4–6, remota, moderate ascendentia, subaequalia vel 1–4 paria distalia minora. Pinnae lineares vel lineari-spatulatae, rectae vel sursum parum curvatae, apice plerumque expansae et dissectae segmentis acutis. Plantae (vivae) cinereo-virides. Textura crassichartacea, rigida. Sporophorum tropophoro 1–2 plo longius.

Fig. 1. Holotype collection of *Botrychium lineare, W. H. Wagner 93039*(OR): Wallowa Co. Lostine River Canyon. Large forms on left, small forms on right. Note that linear segments become branched in the larger forms. Natural size (Cf. Fig. 2).

A small *Botrychium,* the leaf 6–18 cm long above the upright subterranean stem 0.5–3.0 cm long. Trophophore sessile to stalked up to 1 cm. Blade oblong, 1-pinnate. Pinna pairs 4–6, strongly separated by 2–5 times the width of segments, to moderately ascending, approximately equal in length, except for shorter apical 1–4 pairs of segments. Pinnae mostly straight, slightly upcurved, narrowly linear (narrowest in small plants), often expanded at the truncate apex or rarely linear-spatulate, commonly bifid with linear lobes. Color pale green; texture thick chartaceous, rigid. Sporophore 1–2 times the length of trophophore, with a single major axis.

Fig. 2. Variations of *B. lineare* from various localities; c) CO, El Paso Co., Pikes Peak, 2650 m, *W. D. Hauk; J. D. Montgomery & P. Root* in 1991 (private collections); m) MT, Lake Co., Mission Falls, *W. H. Wagner 78549* (MICH); q) PQ, Rimouski Co., St. Donat, Mont Commis, *E. Lepage 3395* (QFA); o) OR, Wallowa Co., Hurricane Creek Meadow, *W. H. Wagner 81128* (MICH). Scale bar = 10 cm.

HOLOTYPE: USA: Oregon: Wallowa Co., Lostine River Canyon, 3.7 miles S of Guard Station 15.6 miles S of Lostine Church, Lapover Ranch between road and river, across road from Belmont cabin, 1600 m, 22 July 1993. *Wagner 93039* and F. Wagner, P. Zika, M. Stein, E.. Alverson (MICH).

OTHER COLLECTIONS: USA: Idaho: Upper Priest River, shaded woods, sandy soil, 3000 m, July 1925. *C. Epling 7457*(LA); Oregon: Wallowa Co., Hurricane Creek meadow W of river, 1.2 miles S of Hurricane Ck. trailhead, 1630 m., 2 August 1981, *W. H. Wagner 81128* (MICH); Montana: Lake Co., Mission Falls, E of St. Ignatius, along woodland trail, very rare, 10 August 1978, *W. H. Wagner 78549* (MICH); California: Inyo Co., Sierra Nevada, W of Bishop Piute Canyon, 1.5 mi. above junction with John Muir Trail, small sloping crevice along trail, highly local 2600 m, 4 September 1968, *L. L. Kiefer 3661(B)* (UC); Colorado: Boulder Co., E slope of Arapahoe Moraine, 1 mi. S of University Camp, gravelly, poorly drained soil, 2900 m, 15 August 1947 *W. A. Weber 3431*(KANU); El Paso Co., road to Independence Pass, Route 82 W from Twin Peaks, Pikes Peak, difficult to see in knee-high grass, 2700 m, mid-July 1989 *Warren D. Hauk s.n.* (MICH); *J. D. Montgomery, Peter R. Root* (priv. herbaria); CANADA: Quebec: Bonaventure Co., Pasepac Lighthouse, gravelly beach, 27 July 1902, *E. F. Williams & M. L. Fernald s.n.* (GRAY); Rimouski Co., St. Donat, Mont Commis, tablette herbeuse au pied de la muraille, 2 July 1942, *E. Lepage 3395*(QFA,MT); New Brunswick: Mountain near Clairs, 11 July 1904, *A. A. Eaton s.n.* (GRAY).

At first the new species might be interpreted as an unusual form, the pinnae of which have become more or less skeletonized, which is an aberrant phenomenon occasionally encountered in other species of this genus. However, on the basis of our present knowledge, *Botrychium lineare* has to be maintained as a separate species. It is uniform and does not change whether it occurs in Oregon, Colorado, or Quebec. It is found in a variety of different habitats without showing modification. It forms populations which, though usually small, may reach up to 45 individuals. It can exist with other species of *Botrychium* nearby, such as *B. crenulatum, B. lanceolatum, B. hesperium,* and *B. minganense,* without showing intergradation. Its morphology does not change from season to season or year to year. Above all, its striking frond characteristics set it apart immediately: 1) the narrowly linear remote segments commonly bifid or more divided at the tips, 2) the nearly uniform size of the pinnae from blade base to near the apex, and 3) the sporophores only 1.0–2.0 times the length of the trophophore. It is one of the more distinctive of the moonworts.

The nearest relative of the slender moonwort may be the widespread and variable *Botrychium campestre* W. H. Wagner & Farrar (1990b) The two species may be keyed out as follows:

1. Trophophore rachis fleshy and broad, 28 (21–33) percent as wide as the whole trophophore. Sporangium mass very dense and fleshy; segments commonly overlapping or fused, linear to spatulate, with 3–5 lobes, the lower segments usually narrowly fan-shaped and lobed, 4–7 x 1.5–2.0 mm. Spores maturing mainly late May and first half of June. Sporophore 1.0–1.5 times the trophophore in length. Exposed dry grassy places at low elevation (ca. 300–1500 m), dunes, plains, and prairies .....*B. campestre*

1. Trophophore rachis more herbaceous and narrow, 17 (14–21) percent as wide as trophophore. Sporangium mass more open and herbaceous. Segments separated, linear to linear spatulate, the largest ones 2 or more times bifid, the lower segments linear, 6–12 x 1.0–2.0 mm. Sporophore 1.0–2.0 times the trophophore in length. Spores maturing mainly late June and July. Deep grass and forbs of meadows, and under trees in woods, and on shelves on limestone

cliffs mostly at higher elevations (ca. 1500–2000 m) in mountains
.....*B. lineare*

To describe a typical habitat for this species would be problematic because the known sites are so different. Our first acquaintance with this moonwort was in 1978 along the banks of a steep woodland trail at Mission Falls, in the Mission Mountains, east of St. Ignatius, Montana. There were only two plants. We later encountered it in the Hurricane Creek Meadows in Wallowa County, Oregon, where two plants were found in a grassy area under a spruce tree. We did not ourselves visit the Pikes Peak, Colorado, locality discovered by Warren Hauk in 1989, but according to his notes the plants were difficult to see in knee-high grass on a hillside; by parting the grasses he was able to find 45 plants, still the largest population we know of. In 1990 we visited Lepage's Mont Commis locality near St. Donat, Quebec (six plants in QKA), to which we were guided by Dr. Pierre Morisset. This extraordinary site comprises a steep limestone cliff with narrow grassy horizontal terraces. Dr. Morisset climbed up and examined the appropriate spot but found only *B. minganense,* which Lepage had also found growing with *B. lineare.* On the ledges of the north-facing steep cliff grow especially woody species such as *Acer spicatum, Betula papyrifera,* and *Thuja occidentalis,* plus a number of herbs, including *Allium schaenoprasum, Calamagrostis lepageana, Carex eburnea,* and *Saxifraga aizoon.* In the rocks at the base of the cliff are such ferns as *Asplenium trichomanes-ramosum* (syn. *A. viride*), *Polystichum lonchitis,* and three species of *Dryopteris.* The fact that we failed to encounter any individuals of *B. lineare* may be due to either of two factors: we visited the locality nearly a half-century after Lepage had collected it. During such a time span, the species could well have disappeared. Or, on the contrary, it may still be there, but did not appear the year we were there. More recent evidence indicates that populations of moonworts often vary greatly in the number of fronds they send up in successive years.

The Oregon site for *Botrychium lineare* discovered in 1992 by Ed Alverson and Peter Ziga in the Lostine River Valley was used by us as the type locality. It is different from the other localities noted above. At the time of our visit, there were 14 recognizable individuals, but the majority had been heavily grazed by some animal. The actual number of plants there may be greater. The upland area in which they grow is approximately 10 x 10 meters and fairly flat. Other botrychiums here are frequent, including *B. lanceolatum* (Gmel.) Rupr. and *B. pinnatum* St. John, but most closely associated is *B. crenulatum* W. H. Wagner. The conspicuous trees here are *Abies concolor, Picea engelmanii,* and *Pinus contorta. Vaccinia* and *Symphoricarpus* are the most abundant shrubs. The herbs include the following genera: *Calamagrostis* and other grasses, *Fragaria, Gentianella, Ranunculus, Rudbeckia* and *Veronica.*

Every effort should be made to preserve the Pikes Peak and Lostine Valley populations of *B. lineare,* which is an extremely scarce and still very poorly known moonwort. The fact that it is found practically across the continent, in Oregon, Colorado, and eastern Quebec, does not alter the conclusion that it is a rarity deserving conservation efforts. Probably not more than 85 plants have been noted thus far in the whole of North America.

ACKNOWLEDGMENTS

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study of evolution and systematics of *Botrychium* was made possible by NSF grants DEB 800536 and DEB 228202768. The following herbaria have generously provided specimens of the new species described here: GRAY, KANU, MICH, MT, QFA, UC.

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Wagner, W. H.and F. S. Wagner. 1990b. Moonworts *Botrychium* subg. *Botrychium)* of the Upper Great Lakes region, and Canada, with descriptions of two new species. Contr. Univ. Mich. Herb. 17:313–325.

Review

***Trichomanes crispum* L. (Pteridophyta, Hymenophyllaceae) and allied species** by Paulo G. Windisch, Bradea 6:78–117. 1992 [1993].

Achomanes is the largest and most taxonomically complex subgenus of *Trichomanes* in the New World tropics. This monograph concerns the difficult *T. crispum* group, which includes 23 of the more than 30 species found in sect. *Achomanes.* Windisch included *T. crinitum* Swartz, the sole species of sect. *Ragatelus,* in this group because its distinguishing character (frond hairs with a swollen and dark-colored basal cell) intergrades with the hairs found in a few species of sect. *Achomanes* (i.e., *T. delicatum* Bosch, *T. galeottii* Fourn., and *T. micayense* Hieron.). He also included *T. humboldtii* Lellinger, a species that appears to be intermediate between typical species of sects. *Achomanes* and *Feea.* Morton did not treat *T. humboldtii* in his conspectus of the Hymenophyllaceae (Contr. U. S. Natl. Herbarium 38:153–214. 1968), but it would logically be placed in sect. *Homoeotes.* However, it matches sect. *Achomanes* in most characters of indument while resembling species of sect. *Feea* only in its dimorphism. I agree with Windisch in both of these taxonomic decisions.

Stable characters are few in these reduced plants. The key to the species, which relies mostly on lamina indument, stipe alation, involucre shape, and frond size, works very well. Synonyms and their types are given for each accepted species, along with a full description, notes, habitat, elevation, and distribution, and representative specimens. A few names remain unplaced, as is usual when dealing with missing or obscure types. Windisch examined and annotated material from more than 30 herbaria in preparing his treatment, and so authentically named material is available in most major herbaria of America, Europe, and South America. All of us who have struggled to identify these interesting plants will have a much easier time of it in the future, and we are grateful for this excellent account.

Mention must be made of the publication date of this paper. Although the cover date is 10 Aug. 1992, copies were distributed in Brazil and to the author in June 1993. — David B. Lellinger, U.S. National Herbarium MRC-166, Smithsonian Institution, Washington, DC 20560.

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Asplenium chihuahuense (Aspleniaceae, Pteridophyta), an Allohexaploid Species and the Description of a Simplified Hybridization Technique

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Asplenium chihuahuense Baker is a rare taxon, probably endemic to north-western Mexico (Chihuahua, Durango). Baker (1891) thought it was *"allied to A. fontanum"*, but in reality it is much closer to *Asplenium adiantum-nigrum* L. McVaugh (1957) and Knobloch & Correll (1962) treated *A. chihuahuense* as a synonym of *A. adiantum-nigrum.* We obtained living material of *A. chihuahuense* and demonstrate below that it differs not only in gross morphology (see Figs. 1–11), but also in chromosome number and genetic constitution, from all the forms of the polymorphous *A. adiantum-nigrum.* Shivas (1969) has shown that *A. adiantum-nigrum* is an allotetraploid species that originated by chromosome doubling in the diploid hybrid *A. cuneifolium* Viv. x *A. onopteris* L. (see Fig. 21). Fusion of one gamete of *A. cuneifolium* (Cu) with one gamete of *A. onopteris* (On) gives a "sterile" diploid hybrid (CuOn). The sporangia of this hybrid produce mainly abortive material but also a few diplospores (see similar cases, e.g., Wagner & Whitmire, 1957; Lovis & Reichstein, 1968; Lovis, 1968; review in Reichstein, 1981, 1982). Tetraploid *A. adiantum-nigrum* must have arisen from such diplospores. This must have been a rare event that happened perhaps only once and long ago, because both ancestors are mainly known from central and Mediterranean Europe while *A. adiantum-nigrum* has a nearly cosmopolitan distribution. Though it shows considerable variation in gross morphology all forms have the same chromosome number (2n=144), similar micromorphological characters, and homologous chromosomes as shown by Shivas (1969) for material from Britain, Luxemburg, Kenya, Transvaal (South Africa) and the U.S.A. Sleep (1983, 1985) showed that this is also true for *A. adiantum-nigrum* var. *silesiacum* Milde, the correct varietal name for the European "serpentine form" [var. *corunnense* Christ (1904) sensu Nogueira and Ormonde (1986)]. This notable form (see Fig. 4) that grows on serpentinite reproduces true from spores and mimics *A. cuneifolium* (see Fig. 9-10) in gross morphology (Sleep, 1969, 1983; Rasbach et al., 1986), but is tetraploid. *A. adiantum-nigrum* is rare in Australia, Hawaii, and La Réunion. We were unable to check material from Australia. Living progeny from Hawaii proved to be tetraploid and morphologically similar to European material. The micro-characters of a small collection from La Réunion correspond to those of the tetraploid taxon.

In our continuing world-wide study of all forms of the polymorphous tetraploid *A. adiantum-nigrum* we have now also included the morphologically similar *A. chihuahuense,* as well as material (comprising the type of *A. andrewsii* Nelson 1904) from the U.S.A. (Arizona, Colorado and Utah).

Materials and Methods

Origin of Plants Used in This Study

Apart from American material, only taxa used for hybridization experiments or for illustrations are listed. All TR numbers (without herbarium abbreviations) are in the pri-

Fig. 1. *Asplenium adiantum-nigrum* from La Réunion, ca. 1700 m (rare), *C.R. Fraser-Jenkins 12326* (=*TR-6616*).

vate herbarium of T. Reichstein (Basel). Abbreviations for herbaria follow Holmgren & al. (1990).

A adiantum-nigrum ssp. **adiantum-nigrum** var. **adiantum-nigrum.** — GERMANY: **Baden-Württemberg:** Black Forest, Glottertal, gneiss rocks, ca. 330 m, 13 Jan. 1988, *H.*

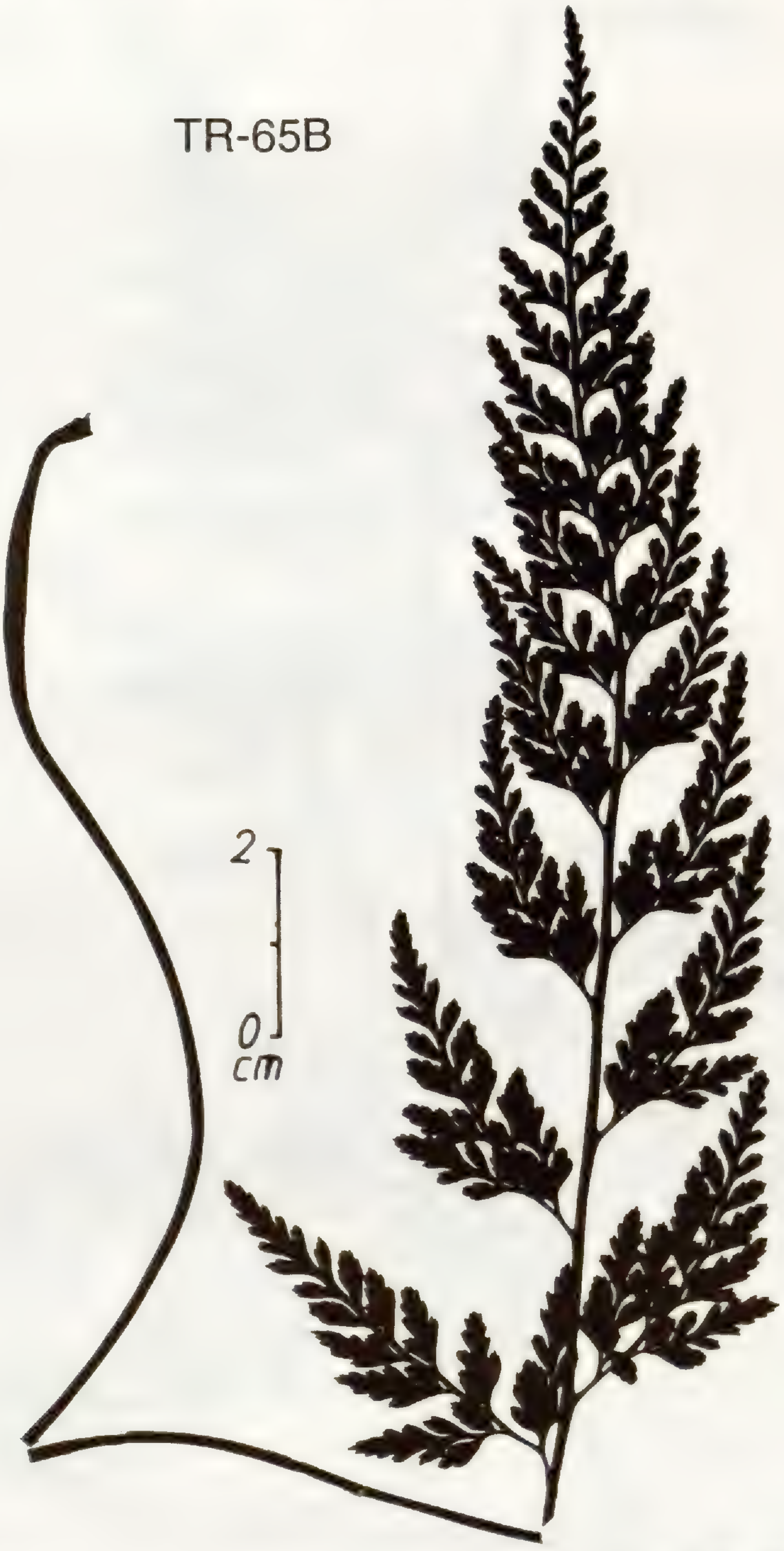

Fig. 2. *Asplenium adiantum-nigrum* from Muzzano, Switzerland, 15 May 1959, TR-65B. Silhouette of pressed frond.

Rasbach s.n.(=*TR-6966*, colony cytologically checked by H.R.: n=72[11]); same locality, 3 Sept. 1980, *R. Viane 1839* (GENT); ITALY: **Lombardia:** Chiavenna, woodland N. of cemetery, 18 April 1984, *R. Viane 2942* (GENT); RÉUNION: Fôret du Grand Matarum, ca. 3 km above Cilaos, Cirque-de-Cilaos, ca. 1700 m, *C.R. Fraser-Jenkins 12326* (=*TR-6616*, see Fig. 1);

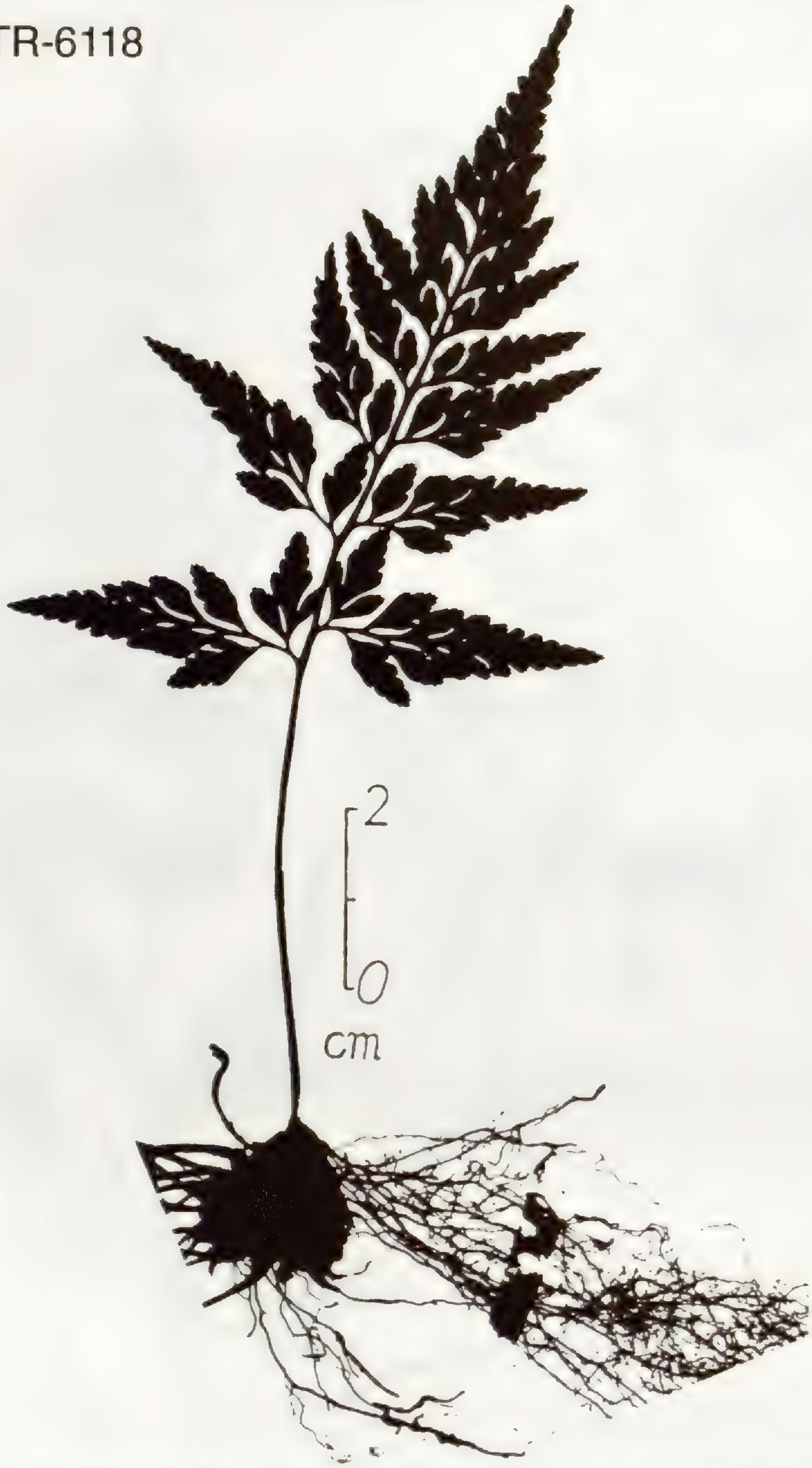

Fig. 3. *Asplenium adiantum-nigrum* cult. in Basel ex spores from Hawaii (*TR-6118*). Silhouette of part of rhizome with one frond.

SWITZERLAND: **Ticino:** near Muzzano, 15 May 1959, *TR-65-B* (Fig. 2); Centovalli, above Verdasio, rock fissures, 810–890 m, 15 April 1984, *R. Viane 2937* (GENT); U.S.A.: **Arizona:** Coconino County, crevices in overhanging cliffs of dacite rock, south face of Elden Mountain, 4 m. NE of Flagstaff, 6 Aug. 1940, *E.T. Wherry s.n.* (=*US 1786865*, US); Flagstaff, Major Brady's School, in rock crevices, mountain slopes, 26 May 1936, *J. Whitehead 2051* (=*US 1726269*,

Fig. 4. *Asplenium adiantum-nigrum* var. *silesiacum:* tetraploid from Mt. Ramazzo (Italy), *TR-4700*. Reproduced from Rasbach et al. (1966:231, Fig. 7).

Fig. 5. *Asplenium adiantum-nigrum* subsp. *yuanum* from Turkey: *TR-3591B*. Silhouette of pressed (1 July 1979) frond after cultivation in Basel, tetraploid.

Fig. 6. *Asplenium chihuahuense:* silhouette of pressed frond, *TR-6827,* raised from spores (in Basel) of *C.R. Fraser-Jenkins 13531,* collected in Mexico.

Fig. 7. *Asplenium chihuahuense:* pinna with sori (drawing: H.R.) of *TR-6827* cultivated in Basel.

Fig. 8. *Asplenium chihuahuense:* isotype, *Pringle 1444,* 15 Oct. 1887 (NY).

US); **Colorado:** Boulder Cañon Cliffs, 1904, *D.M. Andrews 30* (=**holotype** of *A. andrewsii* Nelson, RM); Boulder County, sandstone cliff, *"from type locality"*, no date, *D.M. Andrews s.n.* (=*US691379*, US); Colute Rocks, 7 miles north-east of Boulder, Aug. 1917, *D.M. Andrews s.n.* (=*US1695692*, US); "type locality", 1918, *D.M. Andrews s.n.* (BM); **Utah:** Zion National Park, Sept. 1929, *A.M. Woodbury s.n.* (=*US 1438579*, US); Zion National Park, along nature trail to Canyon Overlook, Jan. 1962, *C. Jepson s.n.* (*US 2639632*, US); **Hawaii:** Maui, Haleakala Crater, terrestrial on cinder cone, 3000 m, 5 June 1984, *A. Piggott 3222* (= *TR-6118*: tetraploid progeny with n=72^{II}, det.: H.R., 25 May 1986; see Fig. 3); no precise loc., cult. Leeds Bot. Garden, cool house, 19 Oct. 1952, *Alston 11842 (=plant 1 from Prof. Fernald*, tetraploid; BM).

A. adiantum-nigrum ssp. **adiantum-nigrum** var. **silesiacum** Milde. — ITALY: **Liguria:** W. of Genova, above Sestri Ponente, Mt. Ramazzo, Miniera, on serpentinite rocks and scree, c. 500 m, 23 July 1978, *G. Martini et al.* (*TR-4700:* tetraploid, n=72^{II}, det.: J. Schneller; see Fig. 4 showing the similarity to the diploid plant of Fig. 9).

Asplenium adiantum-nigrum subsp. **yuanum** (Ching) Reichstein et al. — ETHIOPIA: **Bale:** Saneti plateau and Mt. Batu, 06° 56'N; 39° 40'E, in alpine belt dominated by *Festuca scheffleri, Euryops prostrata,* and *Anthemis tigrensis,* in rock crevices on foot of the highest point of Mt. Batu, ca. 4200 m, 8 Nov. 1982, *I. Kukkonen 12603A* (progeny *TR-5968a:* tetraploid, n = ca. 72^{II}, det.: H.R.). IRAN: **Mazanderan:** Galandrud, limestone, 450 m, 17 Aug. 1970, *Probst s.n.* (=*VO-971*, BSB). TURKEY: **Çoruh** (Artvin): Hopa, rocky slope (schists), c. 9 km E of Hopa on road to Borçka, ca. 600 m, 9 June 1973, *A. & Ch. Nieschalk (TR-3591,* see Fig. 5).

A. chihuahuense (see Fig. 6 to 8). — MEXICO: **Chihuahua:** W. of Chihuahua, ca. 0.5 km SW of road bridge over railway line at S. side of Creel, S. facing calcareous railway cutting below cliffs, ca. 7000 ft. 2 June 1987, *C.R. Fraser-Jenkins 13531* to *13537* [=*TR-6827* (1 to 7), *TR-6828**]; E. side of Creel, above and E. of Children's playground & Parador Hotel, at base of S. facing calcareous rocks, ca. 2300m, 25 Nov. 1992, *C.R. Fraser-Jenkins s.n.*; Creel, edge of south facing cliff, 22 July 1958, *I.W. Knobloch 977* (BM); Sierra Madre, Arroyo Ancho, ledges, 15 oct. 1887, *Pringle 1444* (isotype, NY, See Fig. 8); 10 miles from Rio Tomochic on road from highway 16 (La Junta) to Basaseachic Falls, Pine forest, stream, canyon and rock outcrops (rhyolite?), 6400 ft., 8 June 1978, *T. Reeves, T. Nash, L. McGill & D.J. Pinkava 4877* (GH); **Durango:** Highway 40, 74.5 miles W.S.W. of Ciudad Durango, 10.1 miles W. of El Salta, 1.2 miles E. of Los Adjuntes, Ponderosa Pine-forest, N. and NW. facing slopes with oak understory, boulders with cliffs, ferns in crevices and pits in rock, 8540 ft. (=2603 m), 23°45'N 105°28'W, 15 June 1976, *T. Reeves, T. Nash & L. McGill & D.J. Pinkava 5006* (GH).

Asplenium cuneifolium subsp. **cuneifolium.** — ITALY: **Liguria:** W. of Genua, above Sestri Ponente, Mt. Ramazzo, Miniera (Locus classicus), on serpentinite rocks and scree, c. 520 m, 23 July 1978, *G. Martini, H. Rasbach, T. Reichstein & J. Schneller s.n.* (=*TR-4690*, progeny diploid, n=36^{II}, det.: H.R.; see Fig. 9); SWITZERLAND: **Graubünden:** N. of Davos, Laret, on serpentinite rocks and scree, 1530 m, 13 Sept. 1989, *H. Rasbach RAS-599* (=*TR-6964*, diploid population: Rasbach et al., 1986; see Fig. 10).

A cuneifolium ssp. **woronowii.** — GEORGIA: **Transcaucasus:** W. of Tbilisi, above Borzhomi on Bakuriani road, shale, 1200 m, 23 Aug. 1976, *C.R. Fraser-Jenkins 5560* (= *TR-4028*, progeny diploid, with 2n=72, det.: J.Schneller).

Asplenium onopteris. — ITALY: **Liguria:** sopra Deiva Marina, sul colle di Passano, su serpentina, ca. 330 m, 18 March 1986, *D. Marchetti s.n.* (= *TR-6942:* n=36^{II} det.: H.R. for plant obtained during hybridization experiment TR-7030, Fig. 11); PORTUGAL: **Algarve:** along N124 near Barranco do Velho, dry rivulet bed under *Quercus suber,* 2 July 1979, *R. Viane 745* (GENT); SPAIN: **Cadiz:** Puerto do Ojén, along CA221, ca. 300 m, 18 April 1987, *R. Viane 3523* (GENT).

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* Two clumps of seven nearly dry plants with remnants of green leaves arrived in Basle (25 July 1987), six of which recovered rapidly and became fertile and fixable in Oct. 1987, afterwards a new generation could be raised from spores for hybridization. Fortunately all cytological and hybridization work could be done soon as *A. chihuahuense* proved difficult to cultivate in pots and all mature plants soon died.

RAISING GAMETOPHYTES AND SPOROPHYTES FROM SPORES

Propagation of Taxa. — This was done by T.R. who obtained spores as pure as possible (without any sterilization) by pressing clean mature fronds or leaf fragments in

Fig. 9. *Asplenium cuneifolium* subsp. *cuneifolium* from the locus classicus, Mt. Ramazzo (Italy), *TR-4690*, showing the similarity to the tetraploid taxon of Fig. 4. Reproduced from Rasbach et al. (1966:230, Fig. 6).

clean white paper. These spores were sown, on the Agar medium recommended by Dyer (1979) but slightly modified as described by Rasbach & Reichstein (1990), in Erlenmeyer flasks firmly closed with adherent plastic ("Parafilm M", Amer. Can Comp.) as given in Reichstein et al. (1973). The flasks were kept in full daylight on a S. exposed window-sill at ca. 10–28°C. Green gametophytes usually appeared visible after 4–8 weeks. Fungal or bacterial infections were very rare but could not be avoided completely. We discarded such material and made a new sowing, if required. Slight contamination with mosses or algae could usually be dealt with by pricking out the green prothallia with tweezers, cleaning them several times with water using a tiny brush, and replanting them on fresh agar or soil. When the prothallia reached a length of ca. 2 mm (after ca. 8–12 weeks) they were pricked out and planted in pots (ca. 4–5 cm diameter) in lime-free soil (Rasbach et al., 1983), using a small brush moistened with water and a microspatula. Until this stage these prothallia did not require any particular attention.

Raising Progeny. — ca. 20–50 prothallia where planted in a sterilized pot (ca. 5–6 cm diameter) in lime-free soil, leaving a distance of 3–5 mm between the prothallia. Arrangement for hybridzation: see Fig. 12. The pot was watered immediately, covered with a transparent plastic cup and placed in a temperate, partly shaded greenhouse (ca. 5–28°C, depending on the season), so that the prothallia received some sunshine in the morning and afternoon but were protected from direct sun in summer from ca. 10 to 17 h. They had to be watered carefully every day and kept free of pests. When firmly established (ca. 3–4 weeks after planting) they received their first "bath" to enhance fertilization. For this purpose the outside of the pot was first cleaned with water, whereby sand, algae, etc., were removed, then placed in a clean glass or plastic cup filled with boiled but cooled tap water of 20–25°C up to the rim of the pot. The prothallia were carefully sprayed so that they were just covered with water. The pots were covered with an inverted watch glass and then left for 2 days at ca. 18–22°C in a place where they received bright daylight and full sun during daytime. Afterwards the pot with its plastic cover was put back in the greenhouse. This treatment was repeated at ca. four weeks' intervals until sporophytes appeared. When the sporophytes produced their second or third leaf they were repotted, each in a separate pot ca. 5.5 cm wide and ca. 9 cm high containing a bottom layer of ca. 1.5–2 cm coarse granite grit (ca. 3–5 mm diameter) to secure drainage. After filling the pot with a lime-free soil mixture, the small sporophyte was firmly planted and the soil around it thinly covered with small granite chips. It was watered with tapwater, covered with a plastic cap, and placed on a half-shaded bank in the greenhouse or in a partly shaded sand-bed outdoors in summer.

Cytology

All cytological work was done by H. R. For squashing and preparing permanent slides the technique described by Manton (1950) was followed. For the method to fix immature sporangia in the field or in the laboratory with glacial acetic acidethanol (1:3), some additional details are given in Rasbach & Reichstein (1990). Root tips were pretreated with 0.1% aqueous colchicine, stained with Feulgen reagent and softened by partial hydrolysis in 1N aqueous Hydrochloric acid at precisely 60°C for thirty minutes (Rasbach et al., 1992) and examined by phase contrast with an Olympus BH2 microscope.

MICROMORPHOLOGY

Methods are given by R.V. in Demiriz et al. (1990) and in Viane (1990, 1993). Both for SEM, LM and for exospore measurements only untreated, fresh spores were used. The values of microcharacters (e.g., Table 1 to 3) were obtained from R.V.'s regularly updated database, presently consisting of ca. 90 different collections of *A. adiantum-nigrum* (3758 measurements for exospore size, 3723 for stomata), 6 different samples of

Fig. 10. *Asplenium cuneifolium* subsp. *cuneifolium* from Laret near Davos (Switzerland), *Ras 599 (=TR-6964)*. Silhouette of a frond, pressed 13 Sept. 1989.

Fig. 11. *Asplenium onopteris* from Italy, 18 March 1986, *D. Marchetti s.n.*, (=*TR-6942*), silhouette of pressed frond.

A. chihuahuense (551 & 442 measurements), and 31 different collections of both *A. cuneifolium* (650 & 1320 measurements) and *A. onopteris* (1359 & 683 measurements), all sampled throughout the range of each taxon. SEM perispore terminology follows Viane (1993).

Cytotaxonomical Analysis of Hybrids

The fact that in hexaploid wheat chromosome pairing is controlled by a single gene (Riley & Chapman, 1958) led some authors (e.g., Solbrig, 1968; de Wet & Harlan, 1972) to criticize the technique of genome analysis as it has been applied by Manton (1950) and her school (see also Lovis, 1977: 332). However, in Pteridophytes as well as in Angiosperms, the degree of chromosome pairing remains the most powerful indicator of relationship (e.g., Lovis, 1977; Stace, 1989; Stuessy, 1990).

Manton's Method for Hybridization

The best method for producing experimental hybrids in ferns was worked out by Manton (1950) and orally transmitted to her former students. It was described precisely only by Lovis (1968). In principle, female prothallia are raised in pots in which scattered spores had been sown so that prothallia grow well apart, favouring the production of predominantly female prothallia. These pots were exclusively watered from below. Small, predominantly male prothallia are obtained by very dense cultivation on soil. When microscopic examination shows that the females bear mature archegonia, a suitable number (10–30) are placed on the surface of a ca. 1 cm deep water layer in a petri dish in which many small and narrow, predominantly male prothallia of the other species are submerged. Examination by microscope must show many swimming sperms. The petri dish is kept covered with a watch-glass in full daylight (as bright as possible) for 2–6 hours; the entry of sperms into the open neck of the archegonia can often be observed by microscope. The mostly cross-fertilized, large prothallia swimming on the water layer in the petri dish are then planted in soil in a pot, covered with a plastic cup, and further cultivated in a partly shaded greenhouse and carefully watered daily. Sporophytes, including those of hybrids, usually appear soon and are repotted in individual pots and checked for hybrids when fixable sporangia and mature spores become available. Yields are usually very high, and the method has the advantage that it also permits to establish which of the parents acted as female and which as male parent.

Mainly due to the work of Manton (1950; 1955a,b; 1957; 1958a,b,c), Manton et al. (1986) and many of her former co-workers: Brownsey (1976a, 1976b), Emmott (1964), Gibby (1983), Gibby & Walker (1977), Lovis (1977), Shivas (1969), Sleep (1983), Vida (1972, 1974, 1976), S. Walker (1955, 1961, 1969), T. Walker (1973b), the cytogenetic constitution of most European ferns is well known today. Manton and her former students also investigated many selected groups from Africa, America, and Asia [see Braithwaite (1964, 1986), Ghatak & Manton (1961), Jarrett et al. (1968), Manton & Walker (1953), Manton & Sledge (1954), Manton & Vida (1968), Manton et al. (1966, 1967, 1970), Lovis (1977) and T. Walker (1958, 1962, 1966, 1973a)], while W. H. Wagner Jr. (1954) independently performed pioneer work on natural North American *Asplenium*. Oddly, systematic experimental hybridization has never been used by American workers. The important results of the Manton school were only possible because they had established this reliable method for producing hybrids under experimental conditions, a most remarkable number of which were produced at the University of Leeds.

The above method has one serious disadvantage: it requires a well-equipped institute with suitable greenhouses, a staff of 1 or 2 devoted and well trained gardeners for preparing sterilized soil mixtures, pots, etc., and 1 or 2 assistants for the cytological work. A large number of living prothallia must be available at the correct moment, later a large number of young plants must be attended, repotted, watered, and watched every day over a considerable period.

SIMPLIFIED METHOD

One of us (T.R.) who had started extensive hybridization work (in 1978), did not have access to such facilities. As he had to sterilize Agar-medium and soil mixtures, and make sowings and nurse the plants by himself, he worked out the following, less efficient but simpler method. About 16 immature prothallia of species A are planted in a pot in a square arrangement, and 21 prothallia of species B are placed between them (see Fig. 12). Thus, each prothallium of one species is surrounded by four prothallia of the other, giving a reasonable likelihood for cross-fertilization. The pot is labeled and treated further as described above (raising of taxa). The resulting sporophytes are cultivated to maturity, fixings are made for checking the cytology, and the content of the sporangia is examined to discover hybrids by the presence of abortive spores. This method works well, often with yields of ca. 50%. In the last 16 years more than a hundred hybrids were analyzed to elucidate the auto- or allopolyploid status of their parents, and many new taxa were discovered. Generally, two species possessing a common genome produced hybrids readily. Those between species lacking a common genome were much more difficult to obtain; the yields were often low or nil in the first experiments, but after repetition (2 or 3 times) some important hybrids of this kind could be synthesized. Success in such difficult cases usually depends on the chance that gametophytes of both parents become ready for fertilization simultaneously. This simple method was mentioned by Bennert et al. (1988), but

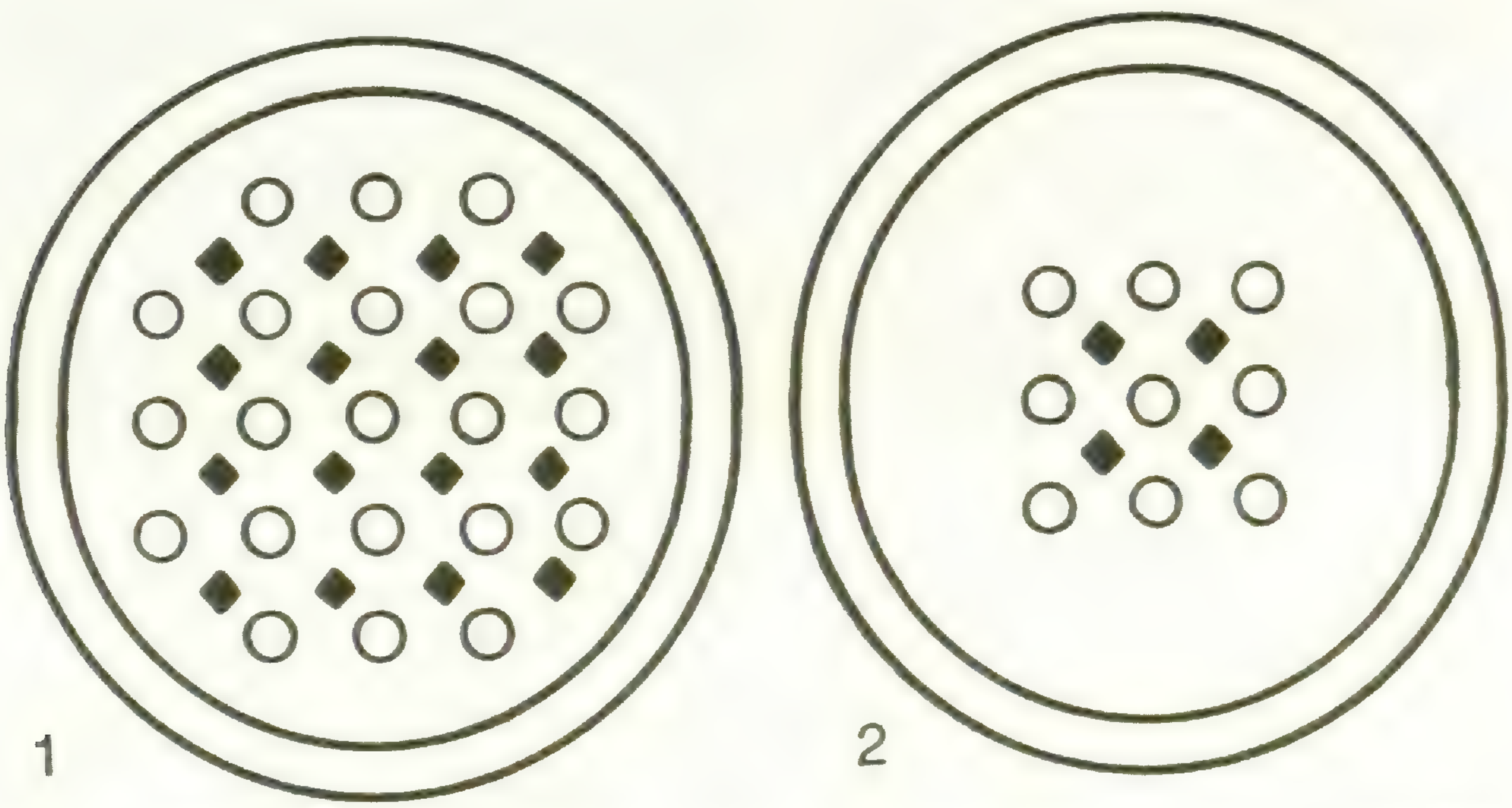

Fig. 12. Planting arrangement for prothallia in hybridization experiments. 1: Experiment *TR-7029*, 2: Experiment *TR-7030*; for explanation see text.

not yet described in detail. We give some more details of the technique in the following two examples:

Hybridization Experiment TR-7029, Performed 11 May 1988 (see Fig. 12). — 16 immature prothallia of *A. adiantum-nigrum* (◆), TR-6966, sown 20 Sept. 1987, were planted in a pot in a square arrangement leaving ca. 8 mm of space between each other; 21 immature prothallia of *A. chihuahuense* (O), TR-6827, sown 20 Feb. 1987, were planted between them (Fig. 12.1). The pot was carefully watered drop by drop, covered with a watch glass and further treated as given above. When sporophytes appeared in sufficient number and produced their second or third leaf they were repotted, each in an individual pot, and grown to maturity. 15 sporophytes reached maturity in July – Oct. 1988. Plants nos. 1, 4, 5, 7 and 15 (=33.3% yield) proved to be pentaploid hybrids (cytology: see below).

Hybridization Experiment TR-7030, Performed 11 may 1988 (see Fig. 12). — Four immature but large prothallia of *A. onopteris* (◆) TR-6942, sown 11 Jan. 1988, and ca. 9 immature prothallia of *A. chihuahuense* (O), sown 20 Feb. 1988, were planted in a pot as shown in Fig. 12.2, and further treated as in the previous experiment. We obtained a total of 41 sporophytes, many more than expected. The reason for this is that the prothallia were transplanted in a very young (partly filiform) state, so that what we assumed to be one single prothallus, must occasionally have consisted of two or three. This did not otherwise affect the result. Of the 41 sporophytes, 18 died in a premature stage. These were probably mostly *A. chihuahuense* which is difficult to cultivate in pots in our region. Among the 23 plants surviving until the summer of 1989 (or partly spring of 1990), plants no. 1, 3, 5, and 12 (= 17.3% yield) were hybrids (cytology: see below). Plant no 2 was *A. onopteris* (n = 36^{II}, with small spores), whereas most of the others were *A. chihuahuense*. It was gratifying that at least 3 reached maturity (although probably not their full size) so that they could be used for illustrating the morphology of this species (see Fig. 6).

Hybridization Experiment TR-7031, (unsuccessful) Performed 11 May 1989 — About 9 immature (filiform) prothallia of *A. cuneifolium*, TR-6964, sown 20 Feb. 1988, and ca. 12 immature (filiform) prothallia of *A. chihuahuense*, TR-6827, sown 20 Feb. 1988, were planted in an arrangement like that of Fig. 12. Soon afterwards the culture succumbed to a fungal infection, and the experiment was not repeated. We mention it to show that such unfortunate events can happen.

Results

Cytological Results

A. chihuahuense. — Meiosis was studied in 7 spore mother cells, the best cell of plant TR-6827 (3), fixed 10 Oct. 1987, showed 108 regular pairs (Fig. 13 A1, B1). Mitosis (2 cells studied) in a root tip of plant TR-6827 (6), fixed 14 Nov. 1987, showed 2n = 216 chromosomes (Fig. 13 A2, B2). These results clearly prove that *A. chihuahuense* is a sexual hexaploid species.

The pentaploid hybrid ***A. adiantum-nigrum x A. chihuahuense*** (Fig. 14.1). Exp.hyb. TR-7029. — The four hybrids 1, 3, 5 and 12 were fixed on 18 July 1989 and 6 Aug. 1989. The 19 spore mother cells studied showed irregular meiosis with n = ca. 68–70 bivalents (perhaps slightly divergent due to the stage of meiosis), the remaining 44-38 chromosomes being univalents. This is very close to the theoretical maximum of n

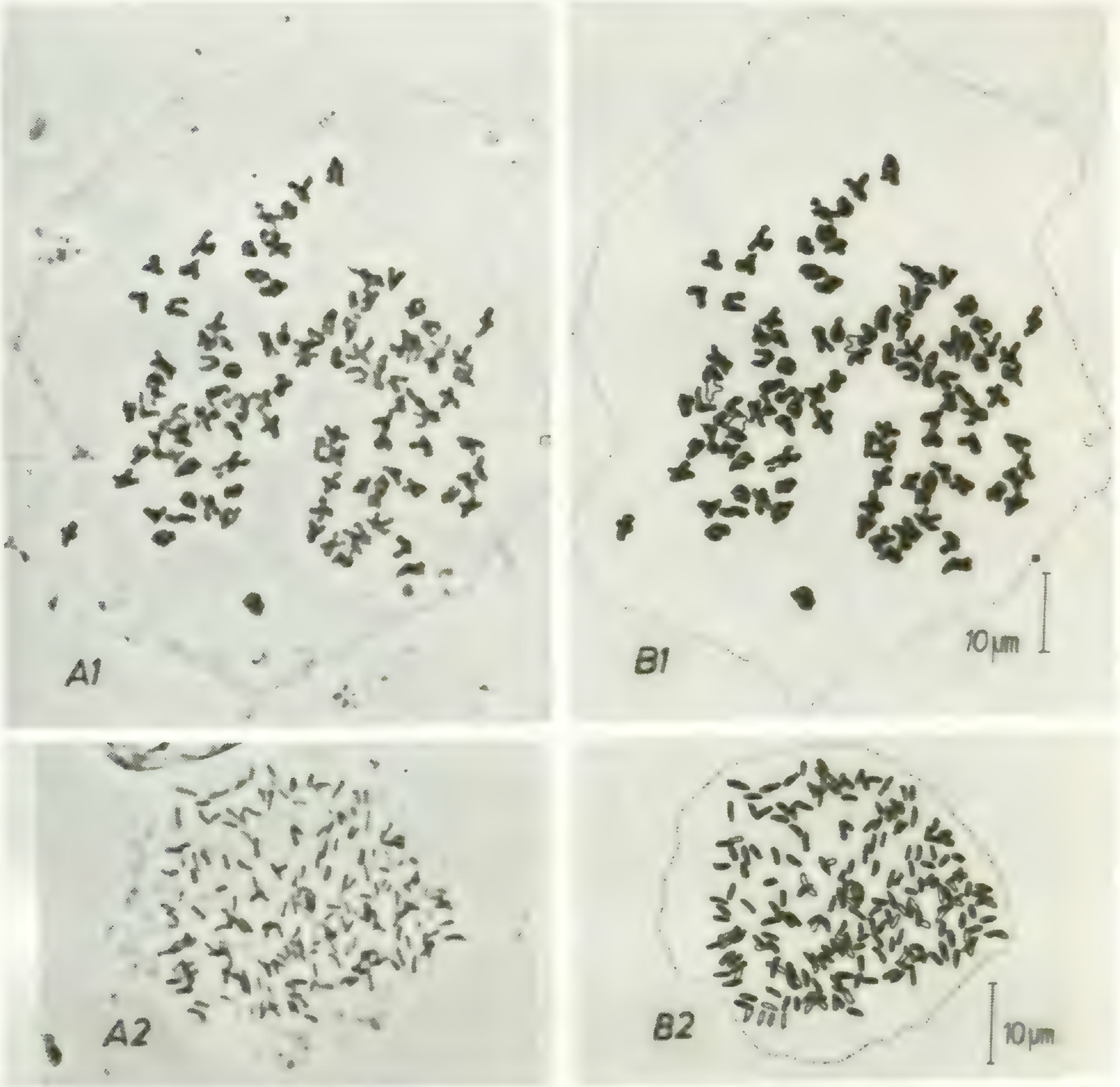

Fig. 13. Cytology of *A. chihuahuense*. *TR-6827(3)* and *TR-6827(6)*. A = photographs; B = explanatory diagrams. A.1, B.1 = Spore mother cell (of plant 3) in meiosis (diakinesis) showing n = 108 pairs. A.2, B.2 = root tips (of plant 6) in mitosis showing 2n=216 chromosomes.

= 72^{II} and 36^{I} for a pentaploid hybrid containing two fully homologous genomes and a single foreign one. This would agree with our hypothesis that *A. chihuahuense* has the genome formula (CuCuOnOnUnUn). This would then lead to the genome formula (CuOnUnCuOn) for the pentaploid hybrid, the four genomes (CuCuOnOn) being responsible for the pairs and the unknown genome (Un) producing the univalents. Fig. 15A1,B1 (photograph and explanatory diagram) shows the analysis of a spore mother cell in plant no. 1.

The tetraploid hybrid ***A. chihuahuense* x *A. onopteris*** (Fig. 14.2). Exp.hybr. TR-7030. — The four hybrid plants 1, 3, 12 and 14 were fixed on 19 June 1989 and 18 July 1989. The 11 spore mother cell studied showed irregular meiosis with n = ca. 36^{II} and 72^{I}. Fig. 15A2,B2 shows one spore mother cell with exactly this number. This result fits very well with the assumed hybrid genome formula (CuOnUnOn) in which (OnOn) would

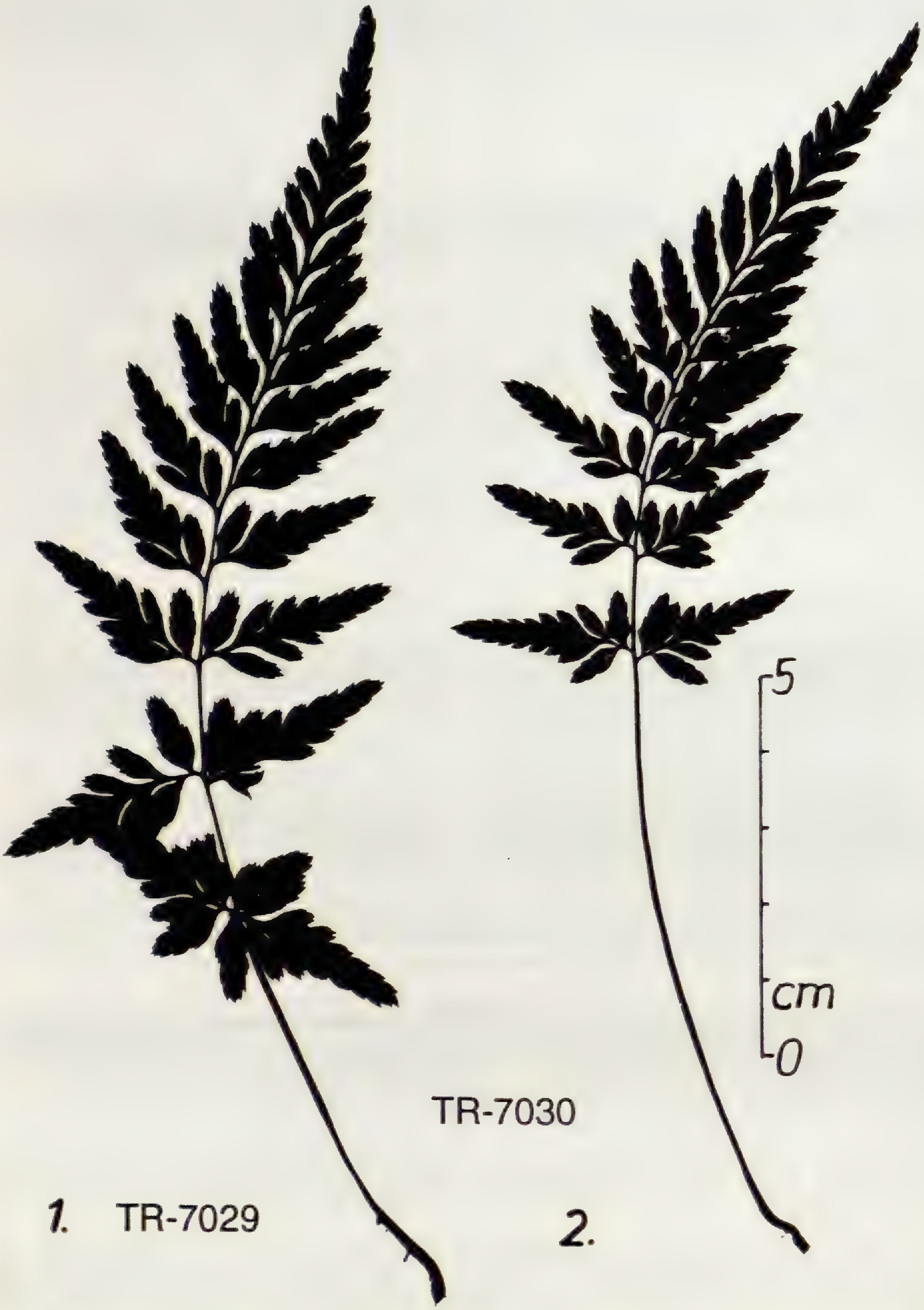

Fig. 14. Silhouettes of a pressed leaf of experimental hybrids. 14.1. Pentaploid experimental hybrid: *TR-7029(1)* = *A. adiantum-nigrum* subsp. *adiantum-nigrum* x *A. chihuahuense*. 14.2. Tetraploid experimental hybrid: *TR-7030(1)* = *A. chihuahuense* x *A. onopteris*.

give the 36 bivalents and (Cu, Un) the 72 univalents.

MICROMORPHOLOGICAL RESULTS

Micromorphological characters of *A. adiantum-nigrum, A. cuneifolium* and *A. onopteris* will be analyzed in detail elsewhere (Reichstein et al., 1994; Viane & Reichstein, 1994). This section will concentrate on their relationships to those of *A. chihuahuense.*

Leaf Epidermis: Cell Pattern and Stomata Size. — The dorsal leaf epidermis of *A. chihuahuense* (Fig. 16.C) is very similar to that of *A. adiantum-nigrum* (Fig. 16 D,E) and *A. cuneifolium* (Fig. 16. B), (see Table 1). Apart from significant size differences of its stomata and subsidiary cells (Table 1 & 3), the former are *acro-* to *mesopolocytic* [anticlinal subsidiary cell walls in contact with guard cells above *(acro-)* or in *(meso-)* the poral region] in *A. chihuahuense,* but rather *meso-* to *basipolocytic* in the other taxa. The anti-

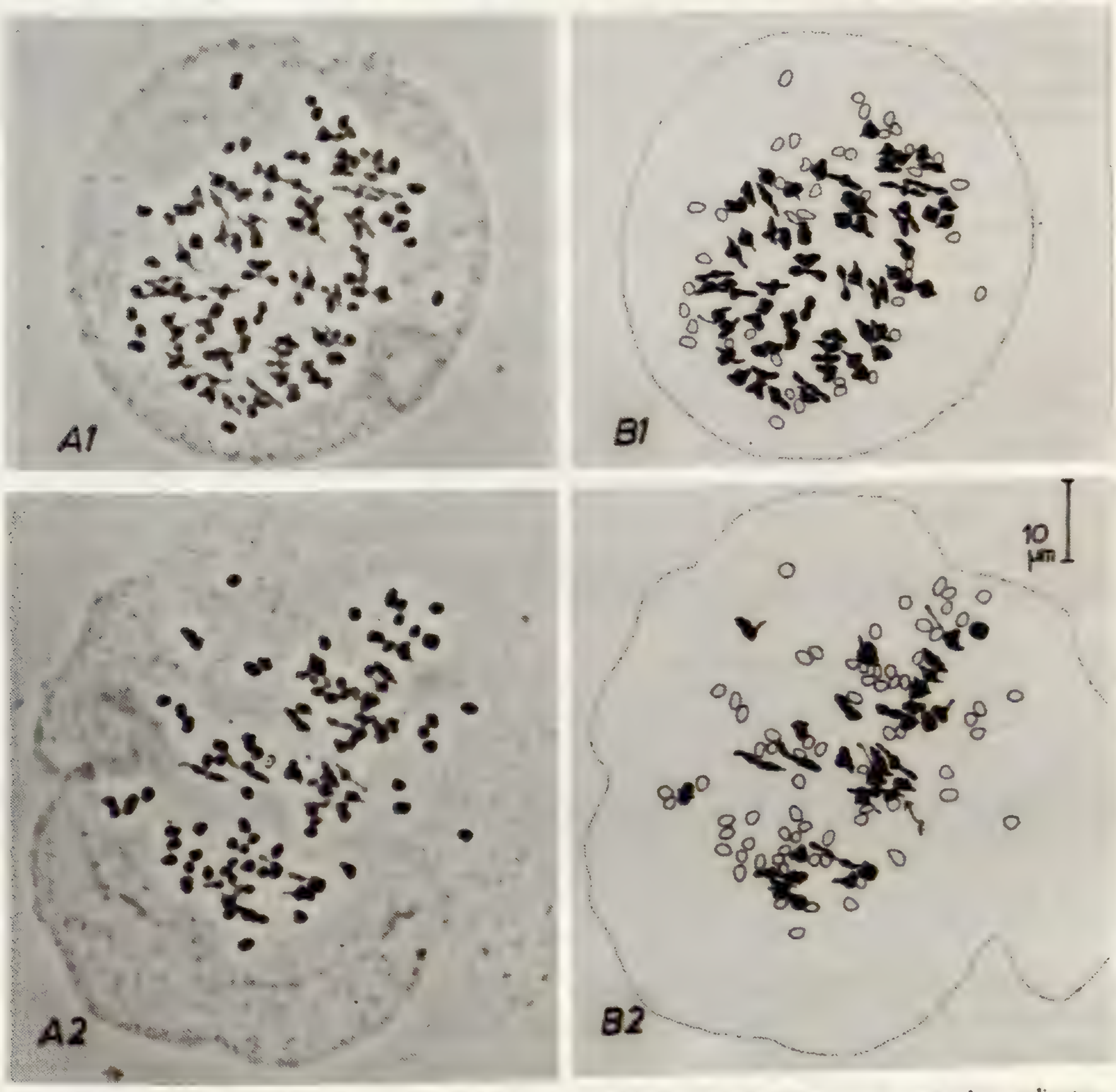

Fig. 15. Cytology of hybrids. A1, B1: pentaploid hybrid *TR-7029(1)* = *A. adiantum-nigrum* subsp. *adiantum-nigrum* x *A. chihuahuense*. Spore mother cell in meiosis (metaphase 1) showing $n=69^{II}, 42^{I}$, total 180 chromosomes. A2, B2: tetraploid hybrid *TR-7030(3)* = *A. chihuahuense* x *A. onopteris*. Spore mother cell in meiosis (metaphase 1) showing n = 36 pairs and 72 single chromosomes, total 144, pairs shown in black, univalent chromosomes is outline.

clinal cell walls of the dorsal epidermis (Fig. 16) are more often bisinuous in *A. chihuahuense* than in the other taxa which are simply sinuous; ventrally its anticlinal cell walls are distinctly sinuous as opposed to merely curved in the other taxa. These last features of *A. chihuahuense* may be due to epidermal characteristics of the unknown ancestor.

Table 1. Comparison of microcharacters.

TAXON	Lpolo in µm	Wpolo in µm	Lepid in µm	Wepid in µm	INSIN in µm	Luepid in µm	Wuepid in µm
A. adiantum-nigrum	86 ± 20.9	65 ± 8.7	211 ± 49.9	48 ± 6.3	14 ± 2.9	205 ± 62.2	28 ± 4.2
A. chihuahuense	120 ± 18.5	65 ± 6.2	226 ± 26.8	49 ± 1.5	13 ± 0.3	222 ± 37.5	37 ± 6.4
A cuneifolium	72 ± 12.9	62 ± 4.6	144 ± 24.8	50 ± 7.1	16 ± 3.1	144 ± 21.3	30 ± 5.9
A. onopteris	58 ± 4.3	47 ± 3.2	175 ± 34.0	30 ± 3.2	6 ± 1.2	236 ± 38.6	20 ± 2.0

Lpolo, Wpolo: length, width of subsidiary cells; Lepid, Wepid: length, width of dorsal epidermal cells; INSIN: double amplitude of sinuate anticlinal cell walls of the dorsal epidermis; Luepid, Wuepid: length, width of ventral epidermal cells. Values given are the mean ± its standard error.

The mean stomata length (Table 3) significantly differs between ploidy levels and can be used for assigning about 75% of the specimens to the correct level. By combining stomata length with exospore size (see Fig. 17) up to 90% of the material can be identified to the species level.

Leaf Indument. — The leaf surface is not "glabrous" as previously described (e.g., Baker, 1891; Knobloch & Correll, 1962), as uniseriate, multicellular hairs are found mainly on the dorsal epidermis of all taxa studied (Fig. 18). Unicellular glands, scales and paleasters (Viane, 1986) are absent. Although the indument of the taxa studied is very similar and demonstrates the coherence within this group, the apical cell is distinctly larger in *A. chihuahuense* than in *A. adiantum-nigrum* (see Table 2). This is possibly not just a consequence of polyploidization (Viane, 1986, 1990; Rasbach et al. 1989), but may have been influenced by indument characteristics of the unknown ancestor.

Table 2. Uniseriate, multicellular laminar hairs.

TAXON	Lbase in µm	Ltop in µm	Lmean in µm	Ltotal in µm	N of cells
A. adiantum-nigrum	86 ± 11.5	38 ± 8.5	55 ± 13.6	278 ± 48.1	5 ± .7
A. chihuahuense	71 ± 11.1	55 ± 13.7	60 ± 13.4	283 ± 47.7	5 ± .7
A cuneifolium	68 ± 12.4	36 ± 11.5	48 ± 14.5	277 ± 48.1	6 ± .6
A. onopteris	77 ± 10.7	29 ± 6.9	44 ± 12.5	236 ± 38.1	5 ± .4

Lbase: length of the basal cell; Ltop: length of the apical cell; Lmean: overall cell length; Ltotal: total length of hair; N: number of cells per hair. Values given are the mean ± 1 standard deviation.

Indusia. — The free indusial margin is irregularly dentate (never entire) in *A. chihuahuense,* mainly repand (slightly uneven and waved) to irregularly sinuous in *A. adiantum-nigrum* (Fig. 19). Only in *A. chihuahuense* some of the marginal indusium cells occasionally bear a short papilla. Further morphological differences between the marginal indusial cells within the *A. adiantum-nigrum* complex are given in Reichstein et al. (1994) and in Viane & Reichstein (1994). The indusial characteristics of the American representatives of *A. adiantum-nigrum* (incl. the holotype of *A. andrewsii* Nelson) all cor-

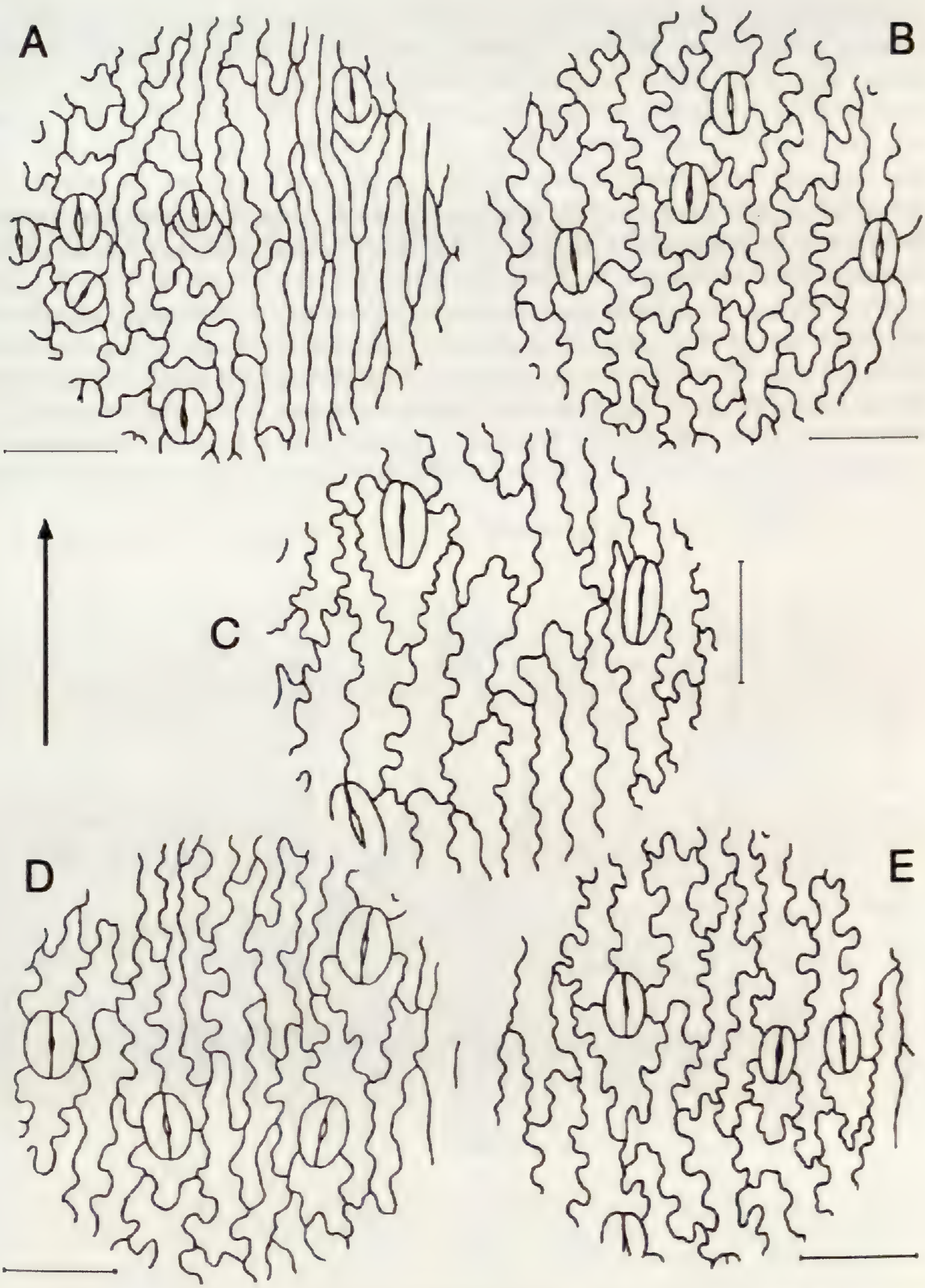

Fig. 16. Cell pattern of the dorsal frond epidermis with stomata. A: *A. onopteris (R. Viane 745)*; B: *A. cuneifolium* ssp. *cuneifolium* (T. Reichstein *TR-4690)*; C: *A. chihuahuense* (Reeves et al. 4877); D-E: *A. adiantum-nigrum* ssp. *adiantum-nigrum* (D: *R. Viane 1839*; E: *D.M. Andrews 30)*. Bar = 100 µm; arrow shows direction of neighbouring veins to the leaf margin.

respond to and fit within the range of variation of the species in its subcosmopolitan distribution.

Perispore. — Morphological differences of the perispore are shown in Fig. 20. As in most other Aspleniums, the *ectoperispore*(= outer perispore layer) is lophate (ridged) in all taxa studied and differences are mainly found in the extent of the areolae (= region between ridges), the perforation density, and the sculpture. The *mesoperispore* (= middle layer connecting the ecto- with the endoperispore) is continuous (= *polymesoperisporous)* in *A. chihuahuense*, restricted to the region below the folds (=*eumesoperisporous)* in *A. cuneifolium* and *A. adiantm-nigrum,* and fluctuates in *A. onopteris.* The (costato-)cristate ectoperispore of *A. cuneifolium* (Fig. 20. 1,3) is almost imperforate and set with very few echinulae in the areolae; in *A. onopteris* (Fig. 20.5) the (cristato-)costate ridges are perforated and the areolae are wider and show more sculpturing. *A adiantum-nigrum* (Fig. 20. 2,4) has an ectoperispore generally intermediate between that of its ancestors. In *A. chihuahuense* (Fig. 20. 6) the cristate perispore folds and (mainly) the areolae are perforated; the ridges have a venulate microsculpture whereas the areolae are densely echinulate. The form of the ridges varies slightly within taxa and cannot be used as the sole diagnostic charater by the casual investigator. The ectoperispore morphology of *A. chihuahuense*

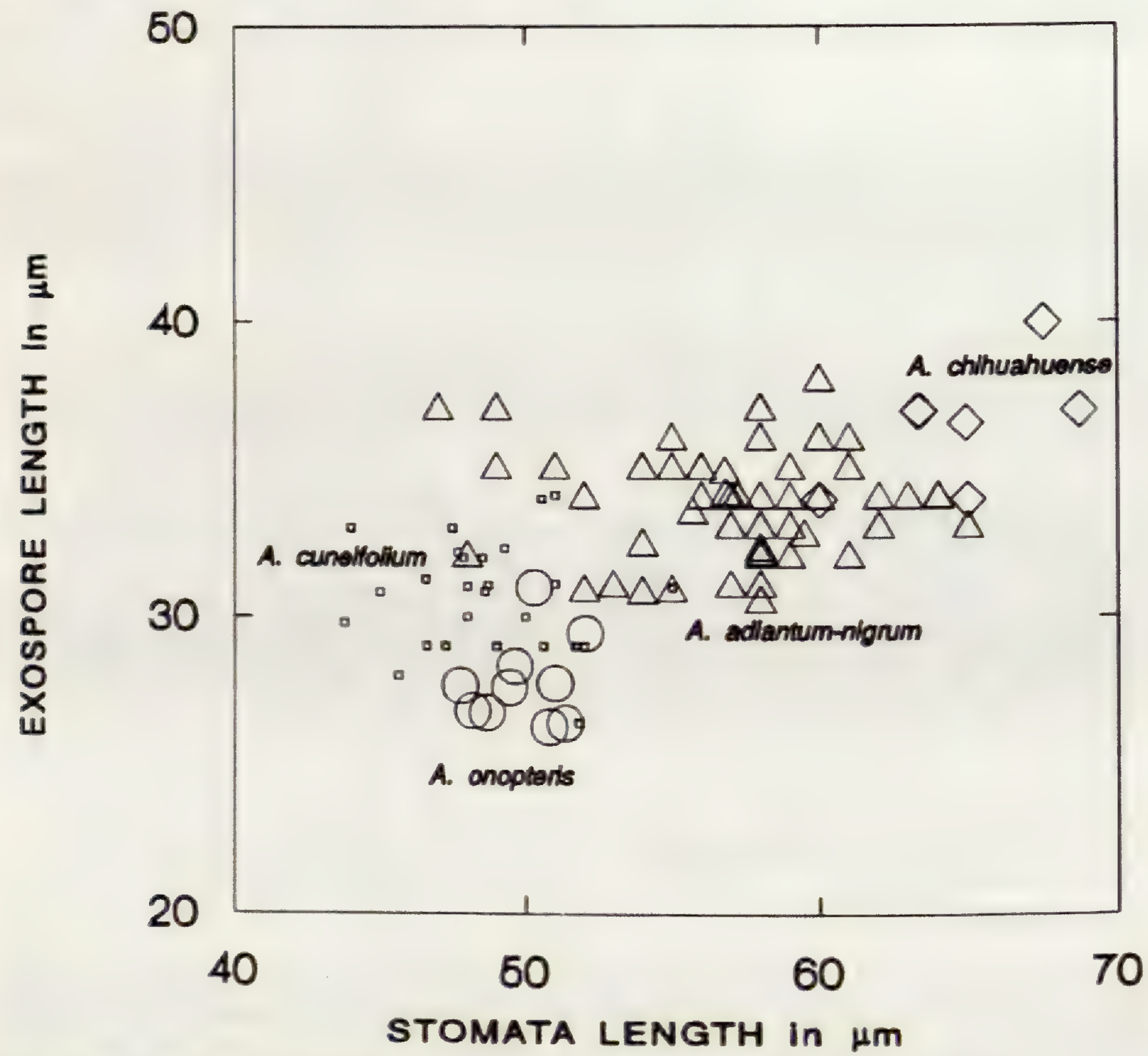

Fig. 17. Plot of mean stomata length on mean exospore length.

confirms its relationship to *A. adiantum-nigrum,* whereas its mesoperispore structure suggests an ancestor with a polymesoperisporous perispore.

Exospore size. — The mean exospore length (Table 3) is significantly different between all taxa and can be used to assign about 70% of the specimens to the correct ploidy level. Combining exospore length with stomata size (see Fig. 17), up to 90% of the material can be identified at the species level; scale length and/or other epidermal microcharacters usually allow the remaining plants to be determined.

Paleae. — The narrowly triangular and typical euclathrate (all anticlinal cell walls evenly thickened, median stripe absent) scales of *A. chihuahuense* are occasionally auriculo-hastate at the base and set with some uniseriate gland-tipped hairs. Though no distinct basal fimbriae (filiform emarginations at least two clathrate cells wide) have been observed, the paleae are similar to those of the other taxa in this complex. Their surprisingly small size (Table 3), when compared to those of *A adiantum-nigrum,* is possibly due to the presence of minute scales in the unknown ancestor.

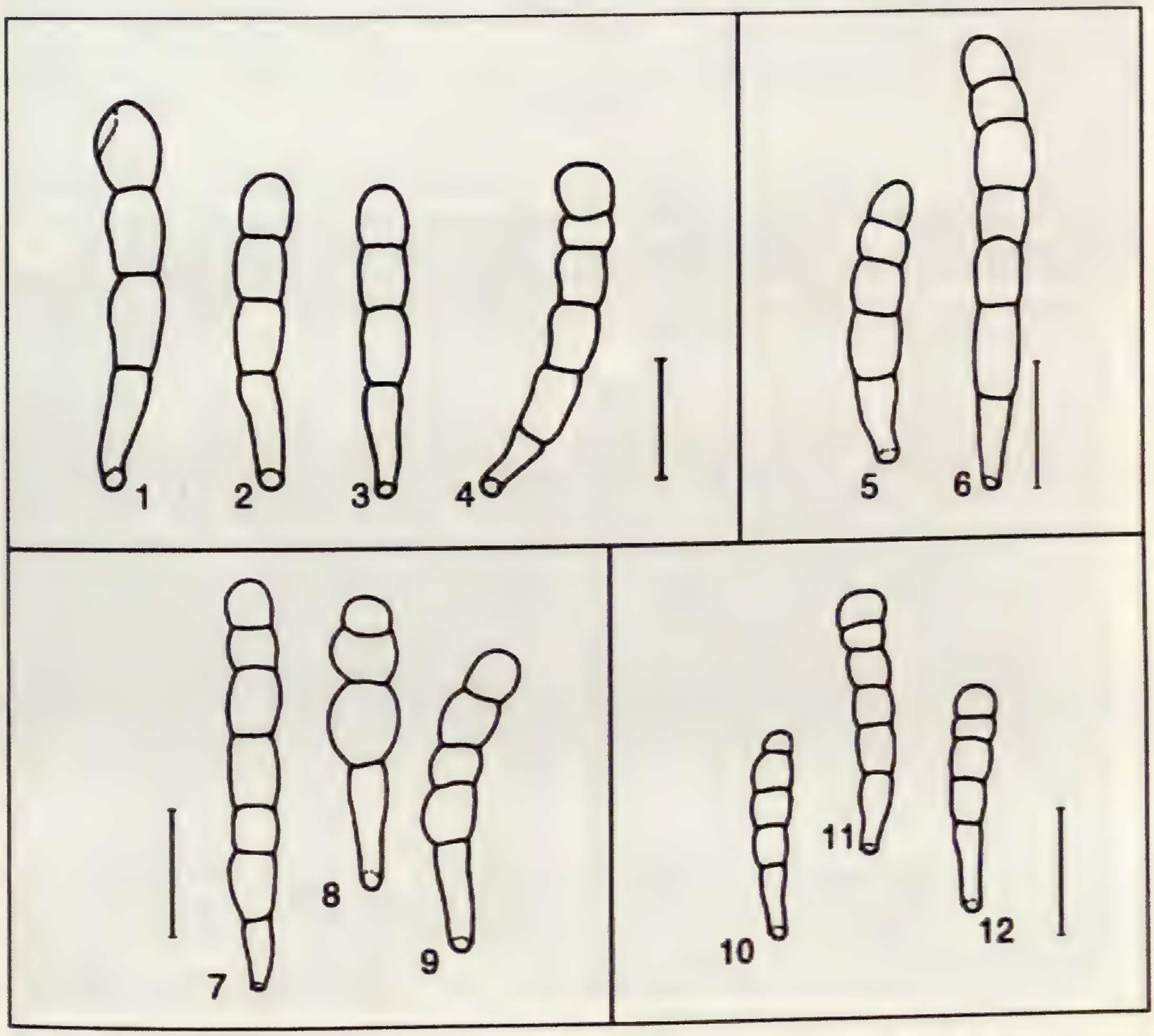

Fig. 18. Uniseriate, multicellular leaf hairs. 1–4: *A. chihuahuense* (1–3: *Reeves et al. 5006;* 4: *Fraser-Jenkins s.n.); 5–6: A. adiantum-nigrum (R. Viane 2937); 7–9: A. cuneifolium* ssp. *cuneifolium (T. Reichstein TR-4690);* 10–12: *A. onopteris (R. Viane 745).* Bar = 100 μm.

Table 3. Comparison of exospore, stomata and scale length.

TAXON	PLOIDY	EXOSPORE LENGTH in µm	STOMATA LENGTH in µm	SCALE LENGTH in mm
A. adiantum-nigrum	4	(21) 31 - **34** - 37 (45)	(32) 50 - **57** - 64 (79)	(1.8) **4.9** ± .77 (7.8)
A. chihuahuense	6	**TR**: (30) 33 - 36 (39)		
		(28) 34 - **37** - 40 (47)	(41) 59 - **64** - 70 (85)	(1.2) **2.7** ± .56 (5.6)
A. cuneifolium	2	(22) 28 - **31** - 34 (44)	(27) 45 - **49** - 53 (66)	(1.5) **2.6** ± .53 (4.5)
A. onopteris	2	(21) 26 - **28** - 31 (39)	(33) 46 - **50** - 53 (64)	(2.4) **5.0** ± .74 (9.6)

Values given are the mean (**bold**) ± 1 standard deviation, and minima and maxima (between brackets). TR: measured by T. Reichstein, all others by R. Viane.

Emended Description

Asplenium chihuahuense Baker, Ann. Bot. 5: 306. (August) 1891 – *Asplenium dubiosum* Davenport, Garden and Forest 4: 483. (October) 1891.

Icon: W. Knobloch & D.S. Correll, Ferns and Fern Allies of Chihuahua, Mexico, 1962: 148, plate 45, figs. 3–4; our Figs. 6–8.

Type: Mexico, Chihuahua, Sierra Madre, Arroyo Ancho, ledges, 15 Oct. 1887, *Pringle 1444* (Holo-: K, temporarily missing, Iso-: NY!), Fig. 8.

In general morphology usually similar to small plants of *A. adiantum-nigrum.* Rhizome erect to ascending, scales clathrate without median band, narrowly triangular, base often widened and with some uniseriate gland-tipped hairs but without fimbriae, 2–3.5 (5.6) mm long. Fronds firmly herbaceous, 5–13 (22) cm long. Stipe castaneous, 2–6 (11) cm long and about as long as the lamina, the base with scales similar to those of

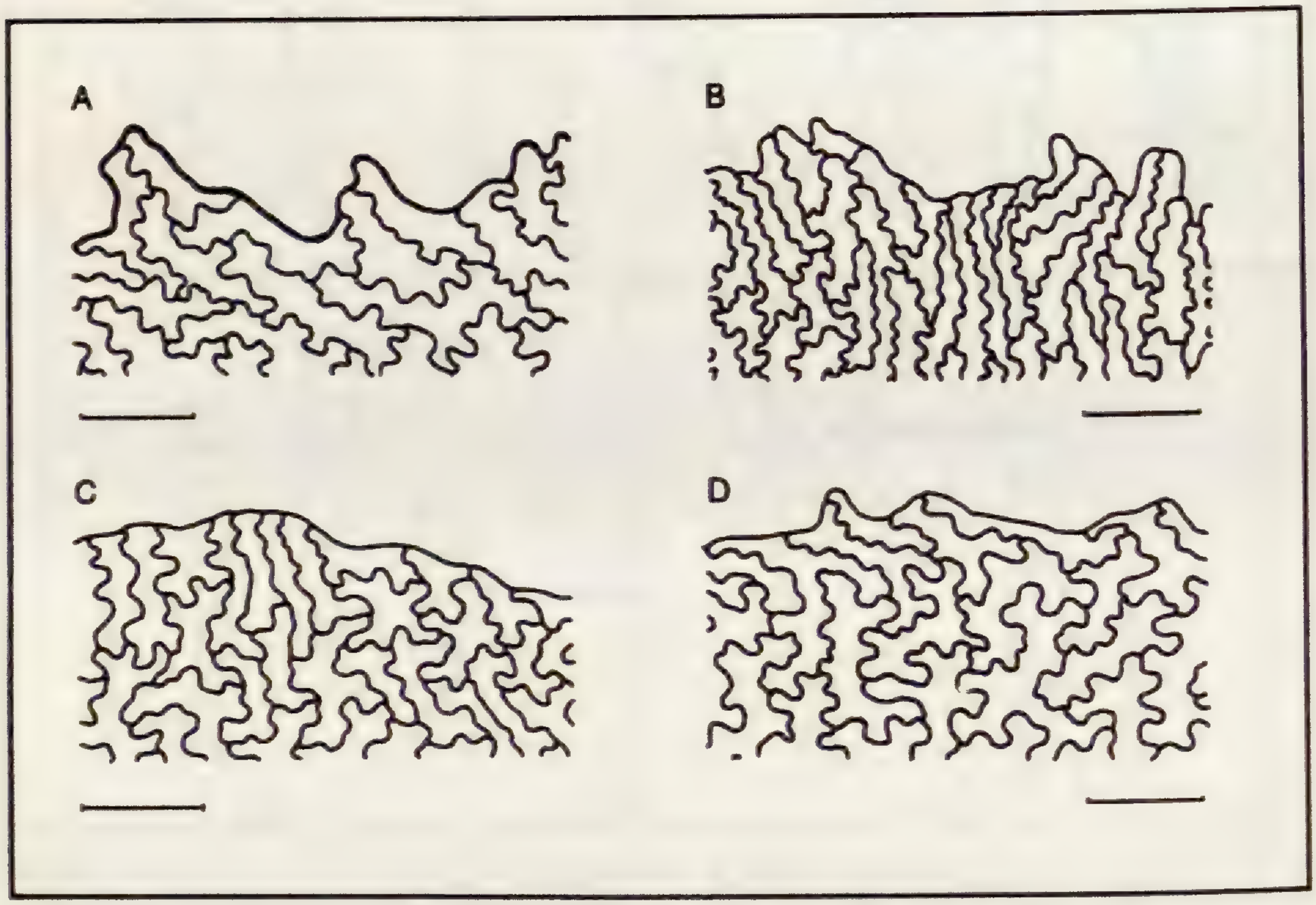

Fig. 19. Margin and cell outline of indusia. A-B: *A. chihuahuense* (A: *Reeves et al. 5006;* B: *Reeves et al. 4877)* C-D: *A. adiantum-nigrum* (C: *R. Viane 2937,* D: *D.M. Andrews 30)* Bar = 100 µm.

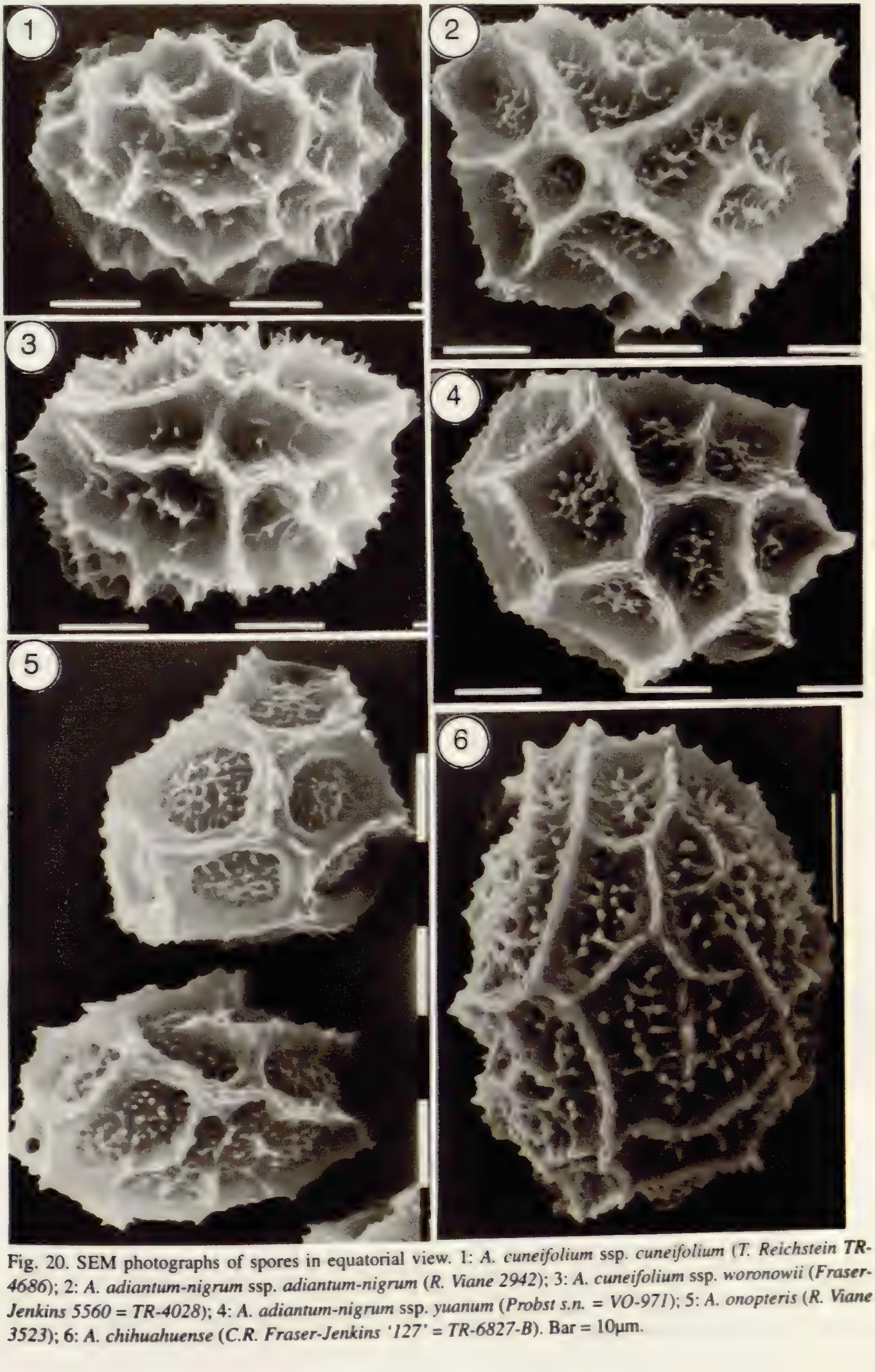

Fig. 20. SEM photographs of spores in equatorial view. 1: *A. cuneifolium* ssp. *cuneifolium* (*T. Reichstein* ***TR-4686***); 2: *A. adiantum-nigrum* ssp. *adiantum-nigrum* (*R. Viane 2942*); 3: *A. cuneifolium* ssp. *woronowii* (*Fraser-Jenkins 5560 = TR-4028*); 4: *A. adiantum-nigrum* ssp. *yuanum* (*Probst s.n. = VO-971*); 5: *A. onopteris* (*R. Viane 3523*); 6: *A. chihuahuense* (*C.R. Fraser-Jenkins '127' = TR-6827-B*). Bar = 10μm.

the rhizome. Lamina deltate to triangular-trullate, 2–8 (11) cm long and 1.5–5 (6) cm wide, 1- to 2-pinnate at the base, large plants exceptionally up to 3-pinnate. Rachis dorsally brown to well above the middle, ventrally green, sulcate (grooved) only at its base and the groove inside without distinct median longitudinal ridge. Pinnae 4–8 (11) per frond, triangular-ovate, 1(2)-pinnate in the basal half of the frond to simple below the apex, only the lower ones stalked (0.5–2 mm), (5) 8–18 (32) mm long and (3) 4–12 (25) mm wide. Basal half of the lowest pinnae with ventrally sulcate costa; costa flat and indistinct apically. Segments triangular to ovate, with cuneate base and obtuse apex; mar-

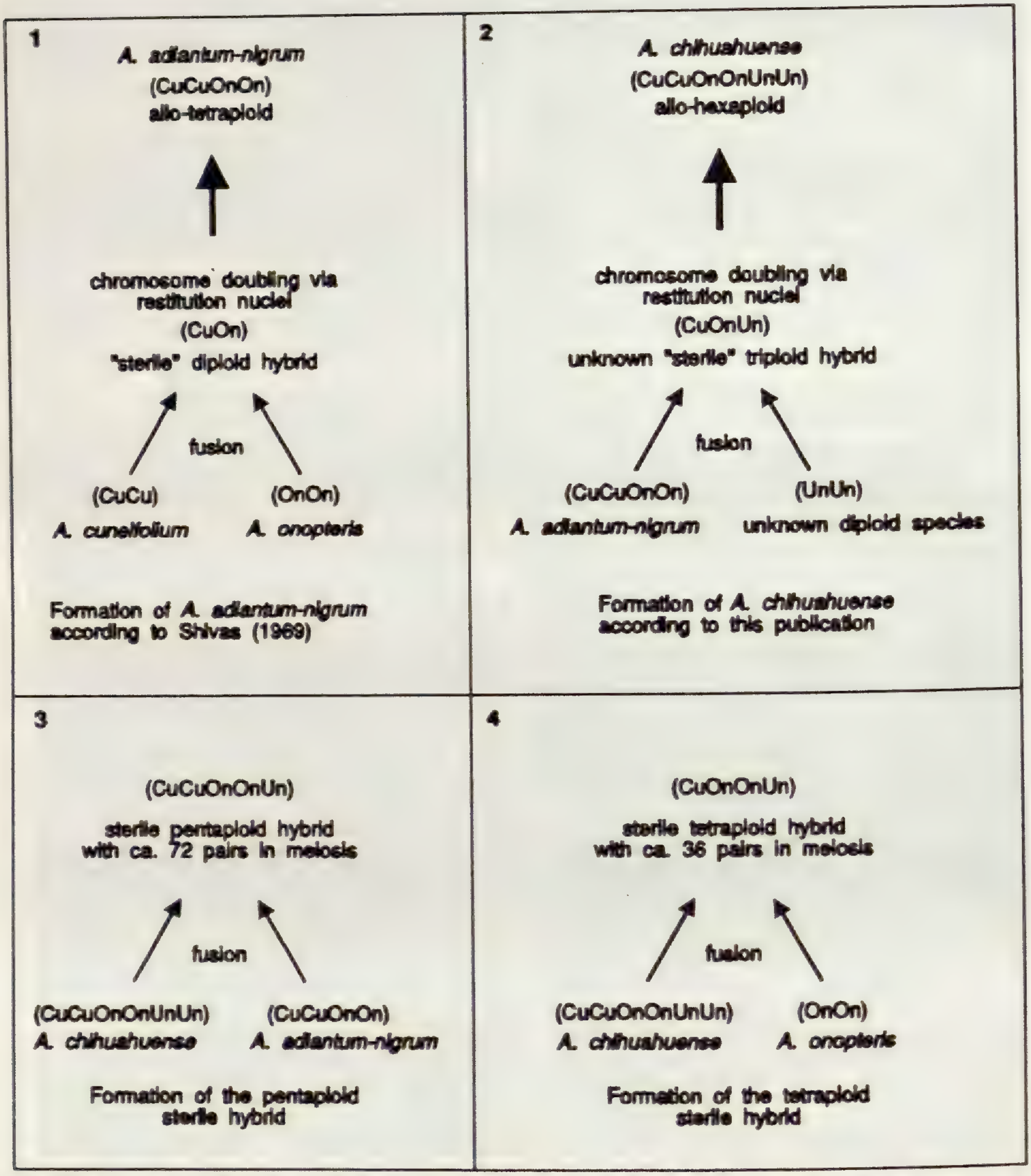

Fig. 21. Charts with genome formulae explaining the formation of the allo-polyploid species *A. adiantum-nigrum* (Fig. 21.1) and *A. chihuahuense* (Fig. 21.2), and the backcrosses of *A. chihuahuense* (Figs. 21.3 and 21.4) with two of its ancestors. Cu = one genome of *A. cuneifolium*, On = one genome of *A. onopteris*, Un = one genome of an unknown diploid species.

gins distinctly serrate. Ventral and dorsal epidermis cells 1-(2) sinuate, stomata polocytic, guard cells (41) 59–70 (85) µm long. Sori basal on the veins, confluent at maturity; indusia irregularly dentate, (0.9) 1.3–2 (2.6) mm long and 0.3–0.5 mm wide. Spores dark brown, exospore (28) 34–40 (47) µm long. Epilithic in crevices of rocks or on roadside cuttings, between 1900–2600 m, so far only in the states of Chihuahua and Durango (Mexico). Hexaploid sexual. For other microcharacters see above (Tables 1–3) and Figs. 16–20.

Small (juvenile) herbarium plants of *A. adiantum-nigrum*, especially those morphologically similar to *A. chihuahuense* (e.g., *Andrews s.n.* p.p., *Wherry s.n.* p.p., *Whitehead 2051* p.p.), can be distinguished by the rachis and costa morphology of the ventral (upper) leaf side. In *A. adiantum-nigrum* both the rachis and the costa groove have a prominent median (longitudinal) ridge and all pinnae, including the distal (upper) ones, have their costa distinctly raised almost to the apex. In large fronds of *A. chihuahuense* the upper side of the rachis is grooved but without a prominent median ridge; in the upper pinnae and in the distal half of the basal (lower) pinnae the costa is flat and inconspicuous.

The living material of *A. chihuahuense* not only allowed us to check its ploidy level, but also to synthesize hybrids with *A. adiantum-nigrum* and *A. onopteris*. We failed to obtain the cross of *A. chihuahuense* with *A. cuneifolium* but the pairing behavior of the chromosomes in the two successfully obtained hybrids shows that *A. chihuahuense* has the genome formula (CuCuOnOnUnUn) in which (Un) stands for the genome of an unknown diploid species. Our results show that *A. chihuahuense* most probably arose by doubling of the chromosomes in a "sterile" triploid hybrid (CuOnUn) between *A. adiantum-nigrum* (CuCuOnOn) and an unknown diploid species (UnUn). See scheme of Fig. 21.

Conclusion

In its gross morphology *A. chihuahuense* fits the range of variation of the cosmopolitan, tetraploid *A. adiantum-nigrum*. It is, however, an allo-hexaploid species with genome formula (CuCuOnOnUnUn) in which (Un) represents the genome of a still unknown ancestor. The pairing behaviour of chromosomes in the experimentally produced pentaploid *(A. adiantum-nigrum* x *A. chihuahuense)* as well as in the tetraploid *(A. chihuahuense* x *A. onopteris)* backcross are in agreement with this postulated formula. *A. chihuahuense* most probably arose locally from a cross of *A. adiantum-nigrum*, which is rare in the SW. U.S. (Arizona, Colorado and Utah), and this unknown second ancestor. This was probably a rare event and may have happened only once. So far *A. chihuahuense* is only known (rare) from Mexico (Chihuahua and Durango). Although it has (incorrectly) been treated as a synonym of *A. adiantum-nigrum* (=*A. andrewsii* Nelson) by some American authors (e.g., McVaugh, 1957; Knobloch & Correll, 1962), *A. chihuahuense* should be regarded as a distinct species.

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QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

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MISSOURI BOTANICAL

Size Hierarchies in Natural Populations of *Polypodium polypodioides*

JUN 2 3 1994

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A size hierarchy describes the differences in size among individuals in a population. Size hierarchies are usually measured using the coefficient of variance or a similar parameter, the Gini coefficient (Weiner and Solbrig, 1984). These two parameters both compare the variance to the mean for size. A low variance (relative to the mean) indicates a small size hierarchy, while a large variance (relative to the mean) indicates a large size hierarchy. Experimental analyses have indicated that size hierarchies increase as plant density, and presumably inter- and intraspecific competition, increases (Weiner, 1985). The assumption that increased size hierarchies can be attributed solely to competition is valid only if other factors affecting size hierarchies can be controlled, as in an experimental plant colony. Among natural populations, size hierarchies cannot be attributed solely to competition (Weiner and Solbrig, 1985). Other factors, such as variation in microsite quality, genetic variation among individuals, predation, and age can affect size hierarchies.

A previous analysis of the distribution of size in co-existing local populations of *Diplazium pycnocarpon* and *Deparia acrostichoides* (Hamilton, 1992) revealed that the local population with a greater density of ramets (i.e., *Diplazium pycnocarpon*), had the smaller size hierarchy. The greater size hierarchy for *Deparia acrostichoides* was attributed to a greater effect of spatial heterogeneity and greater genetic variability among ramets. Competition was eliminated as an explanation for the greater size hierarchy among ramets of *Deparia acrostichoides* because spatial autocorrelation analysis revealed that neighboring pairs of ramets tended to be the same size. If competition was the underlying process regulating size distribution, one would expect to see a much greater occurrence of negative correlations for size among neighboring pairs of ramets.

There is a general lack of information regarding the factors affecting variation in size hierarchies among natural populations. This lack of information regarding pattern makes it difficult to develop an explanation, or even a strategy to seek an explanation, regarding the underlying processes. The purpose of this study is to provide information regarding variation in size hierarchies within natural populations. The data collected will reveal greater insight into processes generating size hierarchies, and suggest testable hypotheses regarding the significance of specific processes.

MATERIALS AND METHODS

The organism chosen for analysis was the fern species *Polypodium polypodioides* var. *michauxianum* Weath. This species was chosen because local populations often occur as monocultures, eliminating the effect of interspecific competition. Thus competitive effects reported are effects of intraspecific competition only. *Polypodium polypodioides* is primarily epiphytic, although it can be epilithic or terrestrial. The species ranges through-

out the southeastern United States, from Maryland to Ohio in the north, south to the coast of the Gulf of Mexico. The plant expands laterally via a creeping rhizome. The plant body of *P. polypodioides* is different from that of most clonal plants previously studied. The ramets produced are single, determinate fronds. For example, ramets of *Diplazium pycnocarpon* and *Deparia acrostichoides* consist of clusters of fronds, and are less determinate than the type of ramet produced by *P. polypodioides*. The growth form of *P. polypodioides* also differs greatly from the growth form of plants used in previous studies of intraspecific competition. As a determinate axis, the size of the frond (ramet) of *P. polypodioides* does not change once it emerges. In previous studies of intraspecific competition, the differential growth of a less determinate axis was the focus of analysis.

In this study, each frond is considered as representative of environmental quality at the microsite where it occurs (each frond was thus considered to be a phytometer, as described by Keddy (1989)). The size of a given frond was therefore considered as a fixed indicator of the response of the plant to environmental quality at a specific microsite.

Subsamples of two different local populations of *Polypodium polypodioides* located in Clinton, Mississippi, were mapped. Mapping was accomplished by placing a finishing nail in the tree bark 2 cm from the first ramet to be mapped. Two points were recorded: point A and ramet number one. A finishing nail was placed at the location of ramet number one. Clear plastic fishing line was attached to point A, and drawn past the nail at ramet number one, the next ramet to be mapped. The fishing line formed an angle point (A: ramet 1: next ramet). The next ramet was mapped using this angle and the distance of the ramet from ramet number one. The subsamples were labeled Plot A, Plot B, Plot C and Plot D. Plot A and Plot D were subsamples of a local population growing on the side of an oak tree on the campus of Mississippi College (the epiphytic habitat). Plot B and Plot C were each subsamples of a terrestrial local population. Each subsample was a spatially discrete unit of the larger local population.

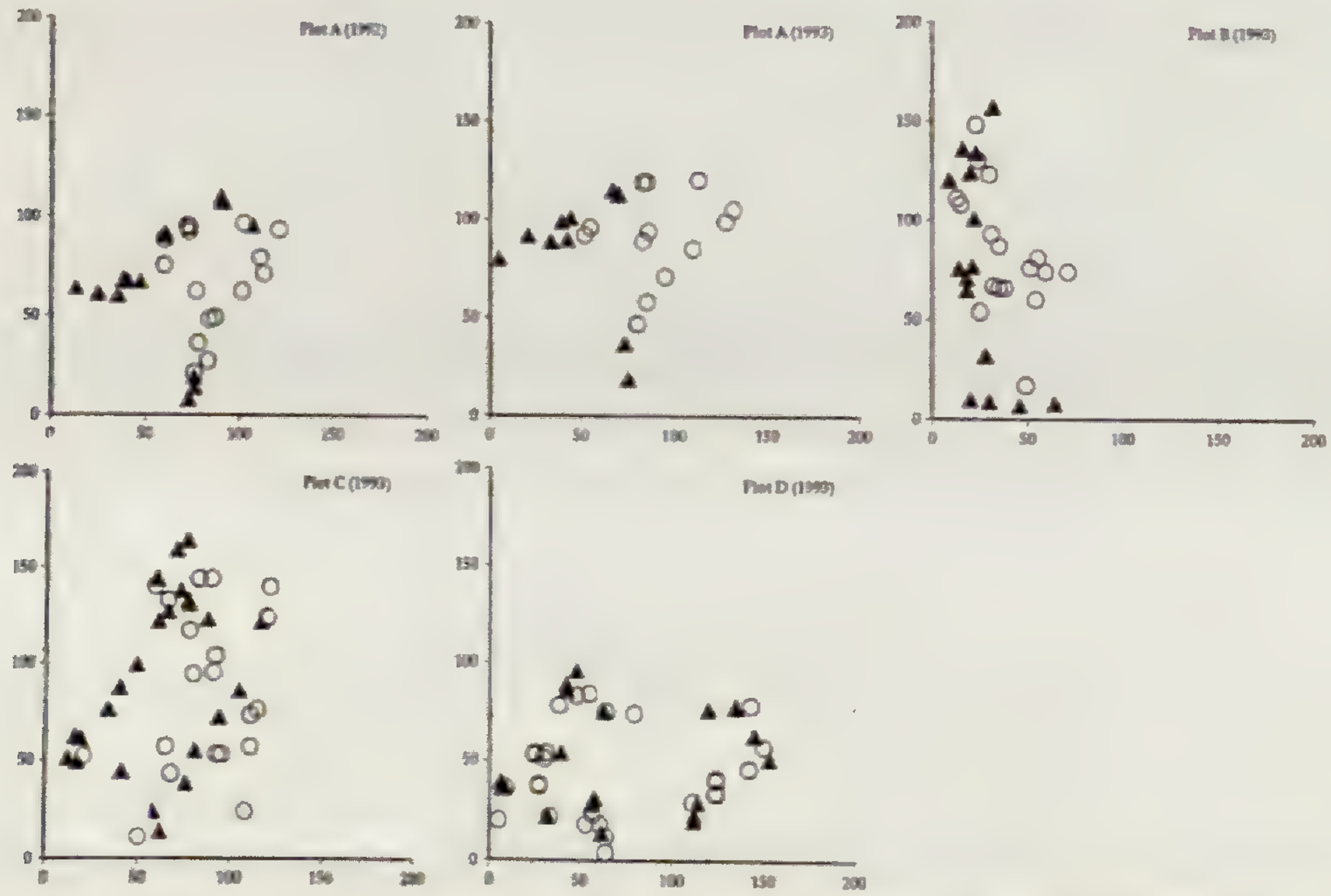

Figure 1. Rectangular coordinate map of ramet locations in each subsample. Triangles indicate ramets larger than average, circles indicate ramets smaller than average.

Ramet sizes were determined by measuring the length and width of the laminar portion of each frond, and using the measures to calculate the area of an ellipse, which was recorded as an estimate of frond size. An estimate of the effect of ramet density was calculated by using the length of each frond as a radius about it's location. The effect of density is expressed as Maximum Potential Interference (MPI), which is the mean number of other points (which indicate ramet locations) within the radii described above. This method allows a consideration of both ramet size and density in estimating the potential effects of density. The observation that increased density leads to increased interference is well established (Harper, 1977). This method underestimates the value of the parameter for Plot B, Plot C, and Plot D because the radii of some points at the edges of the subsample included points outside the subsample, and thus not recorded. All points within all radii were sampled for Plot A.

Spatial autocorrelation analysis (Wartenburg, 1989) was used to determine if there was a non-random distribution of ramet sizes within each subsample. Spatial autocorrelation analysis compares (pairwise) each point to every other point in a population. In our analysis, points were ramets within each subsample, and ramets sizes were compared. If the two ramets of a given pair were either larger or smaller than average, they were considered positively correlated. If one was smaller and other larger, they were considered negatively correlated. The population of pairs of points were divided into distance classes (distance separating the points compared). We used a default setting of 10 distance classes, with 10% of the pairs in each class. We focussed particularly on the smallest distance class. If the points closest together tended to be positively correlated for size, competition is unlikely as a principle cause of a size hierarchy. We considered that significant spatial autocorrelation among ramets indicates an effect of environmental and/or genetic variability on the distribution of ramet sizes and size hierarchies. The parameter used to measure spatial autocorrelation was Moran's I, which is essentially the sum of the effects of each pair of points recorded for each distance class.

Table 1. Sample sizes (n), mean ramet sizes (cm^2), standard deviations, Gini coefficients (99% confidence intervals in brackets), coefficients of variance (CV), skewnesses, and maximum potential interference (MPI) values (variances in brackets) for each subsample.

Plot/Year	n	Mean	Std. Dev.	Gini	CV	Skewness	MPI
Plot A (1992)	30	5.0	2.1	0.242 (0.170-0.312)	0.419	1.94	5.60 (2.24)
Plot A (1993)	23	4.6	1.8	0.233 (0.154-0.288)	0.397	1.85	3.65 (2.22)
Plot D (1993)	47	5.4	3.6	0.369 (0.264-0.454)	0.674	1.05	7.06 (4.33)
Plot B (1993)	33	11.3	5.3	0.263 (0.166-0.375)	0.474	-1.01	12.42 (6.41)
Plot C (1993)	46	12.6	5.7	0.260 (0.175-0.333)	0.452	-0.06	13.83 (7.69)

RESULTS

Size hierarchies, as measured by both the Gini coefficient and the coefficient of variance did not vary significantly among Plots A, B and C (Table 1). The size hierarchy of Plot D was significantly larger than that of the other plots. The mean sizes of the terrestrial plots (B and C) were much greater than those of the epiphytic plots (A and D). Skewnesses for the epiphytic plots were positive, with Plot A having the largest skewness values. Skewnesses of the terrestrial plots were negative. MPI values were also larger for the terrestrial plots.

Spatial autocorrelation analysis revealed that ramets were spatially autocorrelated for size in Plots A and B, but not in Plots C and D (Table 2). Ramets in the smallest distance class were positively correlated for size only in Plot A. The differences in the locations of ramets larger and smaller than average is obvious from inspection of maps of Plots A and B (Figure 1). The lack of distinct differences in the locations of ramets larger and smaller than average is also obvious from inspection of maps of Plots C and D.

The ranges of ramet sizes in Plots B, C, and D were much larger than that of Plot A (Table 3). Sizes ranged from 0.86 cm^2 to 9.21 cm^2 on Plot A in 1992 and 1.77 cm^2 to 7.66 cm^2 in 1993. In Plot B, sizes ranged from 0.57 cm^2 to 22.62 cm^2. In Plot C sizes ranged from 0.94 cm^2 to 25.13 cm^2; and in Plot D sizes ranged from 0.52 cm^2 to 17.59 cm^2.

Extensive predation (by snails), and evidence of predation was observed in the terrestrial plots. No evidence of predation was observed in the epiphytic plots.

Table 2. Results of spatial autocorrelation analysis. Data for each subsample represent a correlogram. DistBound is the upper distance boundary of the distance class described. Stars indicate significance of Moran's I for each distance class. One star indicates $p < 0.05$; two stars indicate $p < 0.01$.

DistClass	1	2	3	4	5	6	7	8	9	10	CgramProb
Plot A (1992)											
DistBound (mm)	15	27	36	43	49	56	64	72	81	112	
No Pairs	43	44	43	44	44	43	43	44	43	44	
Moran's I	0.43**	0.05	-0.02	-0.20	-0.17	0.07	-0.30*	0.18	-0.24	-0.13	0.005
Plot A (1993)											
DistBound (mm)	18	28	36	45	51	60	68	77	88	129	
No. Pairs	25	25	25	26	25	25	26	25	25	26	
Moran's I	0.44**	0.43**	0.27*	0.20	0.11	-0.26	-.054**	-0.15	-0.29	-0.63**	0.001
Plot B (1993)											
DistBound (mm)	17	23	33	41	52	59	69	79	100	157	
No. Pairs	52	53	54	52	52	53	53	53	53	53	
Moran's I	0.06	0.08	0.06	0.01	0.10	-0.03	-0.04	-0.51**	-0.22	0.17*	0.001
Plot C (1993)											
DistBound (mm)	21	35	46	55	64	76	86	96	111	155	
No pairs	103	104	103	104	103	105	102	104	103	104	
Moran's I	0.08	0.06	-0.01	0.09	0.09	0.02	-0.17	-0.01	-0.20*	-0.19*	0.210
Plot D (1993)											
DistBound (mm)	17	32	44	54	63	73	88	97	113	152	
No. Pairs	108	108	109	109	107	108	108	109	107	109	
Moran's I	0.04	-0.04	-0.16	-0.10	0.06	-0.05	0.06	-0.01	-0.07	0.06	0.527

Discussion

Ramets sizes and MPI values were greater for the terrestrial habitat than for the epiphytic habitat. If ramet size is an indicator of environmental quality, the increased mean size indicates that the terrestrial habitat was a higher quality habitat than the epiphytic habitat. The association of an increased MPI with increased environmental quality indicates that the potential for intraspecific competition is increased with increased habitat quality. This observation generally supports the view of Grime & Hodgson (1987), who suggest that competition increases as habitat quality increases.

Table 3. Ramet size (cm^2) and location data for all subsamples.

Ramet	Plot A (1992)		Plot A (1993)		Plot B (1993)		Plot C (1993)		Plot D (1993)	
	Size	Loc. (x,y)	Size	Loc. (x,y)	Size	Loc. (x,y)	Size	Loc. (x,y)	Size	Loc. (x,y)
1	7.16	(13, 64)	7.21	(21, 92)	22.29	(68, 4)	10.84	(92,104)	4.71	(64, 4)
2	9.21	(25, 61)	7.63	(5, 80)	9.25	(54, 60)	10.89	(91,95)	7.84	(64, 12)
3	5.20	(35, 60)	7.66	(33, 89)	8.29	(60, 72)	10.56	(81, 94)	9.90	(62, 14)
4	8.58	(40, 67)	5.34	(42, 90)	0.95	(71, 74)	12.28	(76, 38)	2.12	(53, 18)
5	8.14	(38, 68)	6.94	(33, 99)	10.05	(55, 81)	18.80	(76,38)	2.12	(53, 18)
6	7.06	(47, 64)	6.68	(44, 101)	0.57	(51, 76)	20.78	(81, 55)	4.33	(57, 23)
7	0.86	(60, 75)	3.77	(52, 92)	14.15	(47, 7)	20.05	(94, 72)	6.09	(56, 28)
8	2.83	(60, 88)	4.55	(55, 96)	2.40	(49, 17)	7.07	(93, 53)	5.92	(58, 31)
9	5.18	(61, 90)	5.87	(67, 115)	18.57	(31, 9)	4.34	(95, 53)	6.13	(114, 29)
10	5.87	(60, 91)	5.01	(70, 113)	12.10	(20, 10)	18.85	(105, 86)	13.19	(112, 20)
11	4.12	(72, 95)	1.88	(85, 119)	12.16	(28, 2)	6.83	(108,24)	6.68	(112, 23)
12	2.76	(73, 92)	3.07	(83, 119)	8.80	(25, 54)	4.49	(111, 57)	0.52	(111, 29)
13	4.15	(77, 62)	4.56	(113, 120)	11.31	(21, 77)	4.71	(111, 73)	3.13	(124, 34)
14	4.49	(87, 49)	3.14	(132, 105)	17.77	(18, 65)	4.71	(115, 76)	2.51	(124, 33)
15	4.67	(84, 48)	3.25	(128,99)	8.25	(32, 67)	9.90	(68,43)	0.94	(124, 40)
16	2.83	(78, 36)	1.77	(86, 94)	10.60	(38, 66)	14.14	(62, 14)	3.07	(124,41)
17	1.87	(83, 27)	3.68	(83, 89)	12.79	(14, 76)	15.32	(58, 24)	0.42	(142, 46)
18	3.30	(76, 21)	2.76	(110, 85)	11.41	(18, 71)	9.85	(50, 11)	6.61	(153, 56)
19	6.53	(76, 19)	4.60	(95, 71)	7.04	(35, 66)	13.47	(42, 44)	5.37	(150, 37)
20	5.89	(76, 14)	2.89	(85, 58)	10.84	(35, 87)	8.06	(21, 52)	7.76	(145, 63)
21	7.15	(73, 8)	2.64	(80, 47)	10.17	(32, 93)	18.02	(16, 62)	3.11	(143, 78)
22	6.79	(91, 107)	5.53	(73, 37)	7.70	(23, 93)	11.75	(17, 42)	10.17	(135, 78)
23	6.41	(90, 109)	6.28	(75, 19)	18.38	(22, 101)	25.13	(17, 49)	7.54	(135, 77)
24	2.76	(73, 94)			2.97	(15, 108)	21.99	(19, 61)	9.74	(120, 76)
25	4.18	(102, 96)			9.42	(13, 111)	15.55	(12, 51)	0.79	(79, 74)
26	6.69	(107, 95)			12.88	(20, 124)	21.99	(34, 76)	0.79	(64, 75)
27	3.43	(111, 79)			10.60	(30, 123)	14.53	(40, 87)	9.19	(63, 75)
28	3.63	(101, 62)			10.81	(24, 130)	15.62	(49, 99)	4.91	(48, 83)
29	3.80	(113, 71)			12.30	(9, 120)	18.85	(118, 122)	1.23	(55, 84)
30	3.53	(121, 93)			22.62	(23, 134)	0.94	(121, 124)	11.00	(42, 89)
31					15.58	(16, 136)	0.94	(120, 125)	7.07	(42, 86)
32					10.60	(23, 148)	8.36	(122, 140)	17.59	(48, 96)
33					17.91	(32, 157)	11.31	(90, 114)	5.28	(38, 78)
34							15.76	(89, 123)	10.17	(39, 54)
35							7.56	(83, 144)	4.15	(31, 54)
36							16.12	(77, 164)	2.94	(30, 51)
37							15.94	(71, 159	3.46	(25, 53)
38							16.22	(78, 131)	3.07	(25,54)
39							15.83	(77, 133)	5.30	(33, 22)
40							15.55	(3, 138)	10.17	(32, 22)
41							12.96	(60, 144)	3.61	(27, 38)
42							8.37	(78, 117)	2.82	(27, 37)
43							15.12	(66, 127)	2.34	(9, 36)
44							12.82	(61, 122)	5.92	(7, 36)
45							6.94	(66, 133)	5.42	(8, 37)
46							8.95	(60, 140)	5.50	(6, 39)
47									0.79	(5, 20)

Measures of size hierarchies were not significantly different for Plots A, B and C, while that of Plot D was significantly larger (Table 1). Lack of significant spatial autocorrelation for size among ramets in Plot D (Table 2) eliminates spatial heterogeneity as a principle cause of an increased size hierarchy. Given the absence of predation within the epiphytic habitat, we have concluded that the increased size hierarchy in Plot D was due to an increased effect of intraspecific competition. Note that Plot D does not have the greatest MPI (Table 1), but does have the greatest MPI/mean ratio. Among plots within the epiphytic habitat, there was a positive association between parameters associated with intraspecific competition (Gini and MPI) and mean size, a parameter associated with habitat quality (Table 1). Further analysis of these relations could decisively indicate a positive association between intraspecific competition and habitat quality.

The larger Gini and CV in Plot D over Plot A is mainly due to the larger range of the ramet size distribution in Plot D. The most striking difference in the ranges is the much larger maximum ramet size in Plot D. The range of ramet size distribution in Plot D is more similar to that of Plots B and C, which have much larger means, and much smaller Gini coefficients. Habitat quality in Plot D (in the absence of intraspecific competition) may be similar to that of Plots B and C. The larger Gini suggests an increased effect of intraspecific competition reduced the mean in Plot D to a value similar to that of Plot A. Thus intraspecific competition appears to reduce the habitat quality in Plot D for all but a few ramets, resulting in a mean size (and mean score using the phytometer method) to that of the lower quality habitat observed in Plot A.

Spatial autocorrelation analysis indicated no significant spatial autocorrelation for ramet size in one epiphytic plot (Plot D), and significant autocorrelation for ramet size in the other epiphytic plot (Plot A). Attributing a non-random distribution of ramet sizes to either spatial and/or genetic variation within the subplots, these data suggest that Plot A is either spatially and/or genetically more heterogeneous than Plot D. However, Plot D may not have been any less heterogeneous, either genetically or environmentally, than Plot A. An increased effect of intraspecific competition in Plot D could have rendered the effect of spatial heterogeneity insignificant. This effect could have occurred by increasing the variability for size throughout the subplot, masking the effect of variation among microsites within the subplot. This effect would be enhanced if the effect of intraspecific competition were greater in higher quality portions of the plot, given that an effect of increased intraspecific competition is an increased variance (relative to the man) and a decreased mean (Weiner, 1985).

There was a distinct difference in the skewnesses of the distributions of ramet sizes between the terrestrial and epiphytic habitats, with positive skewness recorded for the epiphytic habitat and negative skewness recorded for the terrestrial habitat (Table 1). The only observed difference between the two habitats that could account for such a difference was the presence of predation in the terrestrial habitat. Weiner (1993) has reported that predation affects smaller size classes more than larger size classes. Predation, by increasing the probability of death for ramets in smaller size classes, could cause a negative skewness in the distribution of ramet sizes. Differences in skewnesses of ramet size distributions existed within the subplots of the epiphytic habitat, with Plot A having a more positively skewed size distribution than Plot D (Table 1). Assuming a higher quality habitat for Plot D, and associating increased vegetative growth with increased habitat quality, skewness in Plot D may be reduced due to an increased rate of recruitment of

new ramets. If the skewness of the size distribution among newly recruited ramets is lower than that of previously existing ramets (among which skewness has been increased due to competitive interactions as predicted by Harper & White (1974)), then the reduction of skewness can be attributed to differences in environmental quality among subplots.

The value of this survey has not been so much in the decisive determination of causality as it has been in suggesting avenues by which more decisive determinations of causality may be made. Further analysis of size hierarchies among natural populations, followed up with the appropriate experiments, promise to reveal much about the ecology of clonal plants.

ACKNOWLEDGEMENTS

The species studied during this investigation has also been described as *Pleopeltis polypodioides* var. *michauxiana*. We thank J. Weiner for a computer program to calculate the Gini coefficient and D. Magers for adapting the program for our microcomputer.

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Reviews

Ferns to Know and Grow, Third edition, by F. Gordon Foster, 1993. Timber Press, Inc., 9999 S. W. Wilshire, Suite 124, Portland, OR 97225. ISBN 0-88192-243-X, paperback $22.95 + 4.00 shipping and handling.

The text is unchanged from a hardcover edition by Timber Press (1984), but the original negatives from earlier editions *(The Gardener's Fern Book,* 1964, D. Van Nostrand Co.; *Ferns to Know and Grow,* 1971, Hawthorn Books, Inc.) have been used to produce sharper photographs. There is also a single-page update of the nomenclature. — ALAN C. CMITH, University Herbarium, University of California, Berkeley, CA 94720.

Gardening with Perennials Month by Month, Second edition, by Joseph Hudak, 1993. Timber Press, Inc., 9999 S. W. Wilshire, Suite 124, Portland, OR 97225. ISBN 0-88192-264-1, hardcover $59.95 + 4.00 shipping and handling.

This is a revised, updated, and expanded version of the 1976 book of the same title (reprinted in 1985). Hudak discusses or lists 700+ species, arranged according to their blooming or most showy period (March through September) and further categorized by color and hardiness. Fern enthusiasts will find 12 pages of discussion and photographs, treating 36 commonly grown and aesthetically pleasing species, mostly eastern North American natives. The color photographs (400+) are an outstanding feature of this book. — ALAN C. SMITH, University Herbarium, University of California, Berkeley, CA 94720.

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Quillworts of Virginia

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This paper discusses the taxonomy and distribution of the six species and one hybrid of *Isoëtes* occurring in Virginia. Like most regions, the quillwort flora of Virginia has not been intensively studied because of the cryptic nature of the plants and because of the paucity of characters used to distinguish among species. Virginia has been unevenly collected for quillworts. For example, there are many herbarium sheets of *Isoëtes* from the Mountain Lake region in Giles County in the Allegheny Mountains, from the Big Meadows Swamp along the Blue Ridge Parkway and from Southeastern Virginia, especially the watershed of the Nottoway and Blackwater Rivers. On the other hand, there is only one collection of *Isoëtes* from extreme western Virginia and none from the Eastern Shore (Fig. 1).

Most notable among collectors in southeastern Virginia is the indefatigable Merritt Lyndon Fernald who collected extensively in the 1930's and 1940's and produced numerous publications based on his field work (eg, Fernald, 1937, 1940). Fernald's collections are of especial value in documenting the decline in the distribution and abundance of *I. riparia* in tidal rivers. In addition, he regularly, albeit unwittingly, collected *I. X brittonii*, a hybrid between *I. engelmannii* and *I. riparia*. The collections of A. M. Harvill, Jr. and his collaborators in the Atlas of the Virginia Flora project have added considerably to our understanding of the distribution of *Isoëtes* in Virginia. His work is summarized in Harvill et al. (1992). As a result of these contributions, the number of taxa recognized from Virginia has more than doubled (Table 1).

During the past decade there has been a growing awareness of the frequency of hybridization in the genus *Isoëtes*. Luebke & Taylor (1985) summarized some of the early observations of hybridization. One of the first papers to suggest that hybridization was frequent in Southeastern quillworts is that of Matthews & Murdy (1969) who worked with granite outcrop species. Boom (1980) showed that it was possible to produce hybrids between species from different sections (sensu Pfeiffer, 1922). A review of *Isoëtes* in the Southeastern United States by Boom (1982) drew further attention to hybridization. More recently, several naturally occurring hybrids have been described (Taylor & Luebke, 1988; Britton, 1991; Britton & Brunton, 1989; Brunton & Britton, 1991; Brunton & Taylor, 1990 ; Taylor, 1992). The discovery of hybrids appears certain to continue. To date, only one hybrid has been reported from Virginia (Brunton & Taylor, 1990). In fact, hybridization is probably as common in *Isoëtes* as many other pteridophytes.

Virginia quillwort hybrids are vegetatively indistinguishable from either parent, but readily recognized by spore characters, a conservative feature on which most determination depends (Hickey, 1986). Spores of hybrid plants are variable in size with many broken and malformed spores (Fig. 2). The ornamentation of hybrid spores has a range of expression of both parents.

Table 1. Species of floristic treatments of Virginia *Isoëtes*.

	Pfeiffer, 1922	Fernald, 1950	Massey 1960	Wherry, 1961	Reed, 1965	Boom, 1982	Gleason & Cronquist, 1991	Harvill, et al., 1992	Taylor et al., 1993	Musselman & Knepper, 1994
I. Xbrittonii						X			X	X
I. caroliniana	X	X	X		X				X	X
I. engelmannii	X	X	X	X	X	X	X	X	X	X
I. engelmannii var. *fontana*			X							
I. lacustris			X	X	X	X	X	X	X	X
I. melanopoda				X	X	X	X		X	X
I. riparia	X	X		X		X	X	X	X	X
I. riparia var. *palmeri*			X		X					
I. riparia var. *reticulata*						X				
I. saccharata	X		X							
I. virginica		X	X	X	X	X	X	X	X	X

Material and Methods

This study is based on our field, lab, and herbarium work during the past four years. We have examined Virginia material from the following herbaria: FARM, GH, GM, LYNCH, NA, NCU, OAC, ODU, TENN, US, VPI, and WM. Unfortunately, many specimens are either sterile or have only microspores; these are not cited. Herbarium sheets from freshwater sites in southeastern Virginia containing more than one plant are often mixed collections. Exsicattae with the same number from two different herbaria may contain as many as three taxa. Our collections are deposited at ODU; duplicates have been distributed to VPI and GH.

Descriptions are based on Virginia material except for microspores of *I. virginica (Musselman and Knepper, sn).* We follow the nomenclature of Taylor et al. (1993) and do not repeat synonymy here.

For observation with the scanning electron microscope material preserved in FAA was first critical-point dried. Dried material was not critical point dried but otherwise handled the same way. Specimens were sputtered coated with a gold palladium mixture, approximately 100 angstroms thick, and viewed with a Cambridge 100 Scanning Electron Microscope. No correction has been made for spherical aberration.

Spore measurements followed the protocol outlined in Kott & Britton (1983). Approximately 20 spores from a sporangium were mounted in Euparol and measured using a light microscope equipped with an ocular micrometer (generally 40X for megaspores and 1,000X for microspores). The work of Kott & Britton (1983) indicated that the amount of variability in megaspore size within a single individual was comparable to that between individuals within a single population; therefore, megaspore measurements for a single individual could be considered as representative for the entire population. Based on this assumption, relative spore sizes of Virginia quillworts were determined by measuring spores (N=20) from a single sporangium of an individual plant. For *I. lacustris* and *I. virginica,* a single population was available for each. Mean spore diameter measurements were tested by a Student-Newman-Keuls (SNK) range test to determine if significant size differences were present among populations.

Virginia quillworts are remarkably easy to grow and all taxa have been cultured under uniform conditions for one year or more.

Taxonomic Characters

With some practice, it is easy to recognize quillworts in the field. They are the only plants in our flora with spirally arranged grass-like leaves with a furrow on the adaxial surface of the leaf. Species determination, on the other hand, is a frustrating problem because of the limited number of characters that can be used. Basically, all quillworts superficially look the same and despite efforts to employ a diversity of characters for determination (Boom, 1982) and warnings against constructing taxonomic schemes on single characters (Hickey, 1986), mature megaspores remain the sole reliable means of species determination for most Virginia material. Only with field experience is it possible to determine quillwort *in situ* and even then identification may be difficult. Following is a discussion of the characters we use to describe Virginia quillworts. In this discussion, we are following Kott & Britton (1983) for megaspore terminology: proximal hemisphere, distal hemisphere, equatorial ridge, radial ridges (=tri-radiate ridges or crests). The term equatorial ridge refers to the ridge encompassing the megaspore at approximately the midpoint. Girdle refers to a short region distal to the equatorial ridge with a distinct ornamentation (secondary ornamentation, sensu Hickey, 1986). Descriptive terms for ornamentation are based on Hickey (1986).

HABITAT AND DISTRIBUTION: In Virginia, if the plant is on a rock outcrop, it is either *Isoëtes melanopoda* or *I. virginica.* On the other hand, collections from coastal rivers can be one of three taxa: *Isoëtes X brittonii, I. engelmannii,* or *I. riparia,* and only rarely *I. melanopoda.* The only quillwort to inhabit brackish water is *I. riparia.* In mountain streams and ponds, the most frequent quillwort is *I. caroliniana.* This species can be assumed to be the only quillwort at highest elevations. *Isoëtes engelmannii* is found in a diversity of habitats throughout the state. *Isoëtes lacustris* and *I. virginica* both are known from single stations. Distribution of Virginia quillworts by county is summarized in Fig. 1.

HABIT: All Virginia quillworts, except *I. lacustris* which is always aquatic, can be amphibious, at least when older. Amphibious plants may not be evergreen.

CORM: All of our quillworts have a two-lobed corm but this architecture is altered with age. The shape of the corm is of diagnostic value in Virginia quillworts when plants are older. *Isoëtes riparia* and *I. virginica* have sharply elongate corms. Older corms of *I. virginica* are entirely elongate; the round shape of the young corm is obliterated. Other taxa are round in cross section.

LEAVES: Leaf length, number, pliancy, and to a lesser extent, color are strongly influenced by environment. Leaves from still water have more evident diaphragms than leaves from moving water. The lower portion of the leaf, near the sporangium, is often winged. These wings, or alae, are more pronounced in plants which do not grow in running water. We have grown all Virginia quillworts under uniform conditions and the leaves look remarkably similar. Despite this, there are subtle differences among species. The color of *I. caroliniana* and *I. melanopoda* tends to be lighter than that of other species. The only quillworts with phyllopodia (indurate, darkened sporophyll bases) are *I. melanopoda* and *I. virginica.* Phyllopodia persist at the base of the plant during the dor-

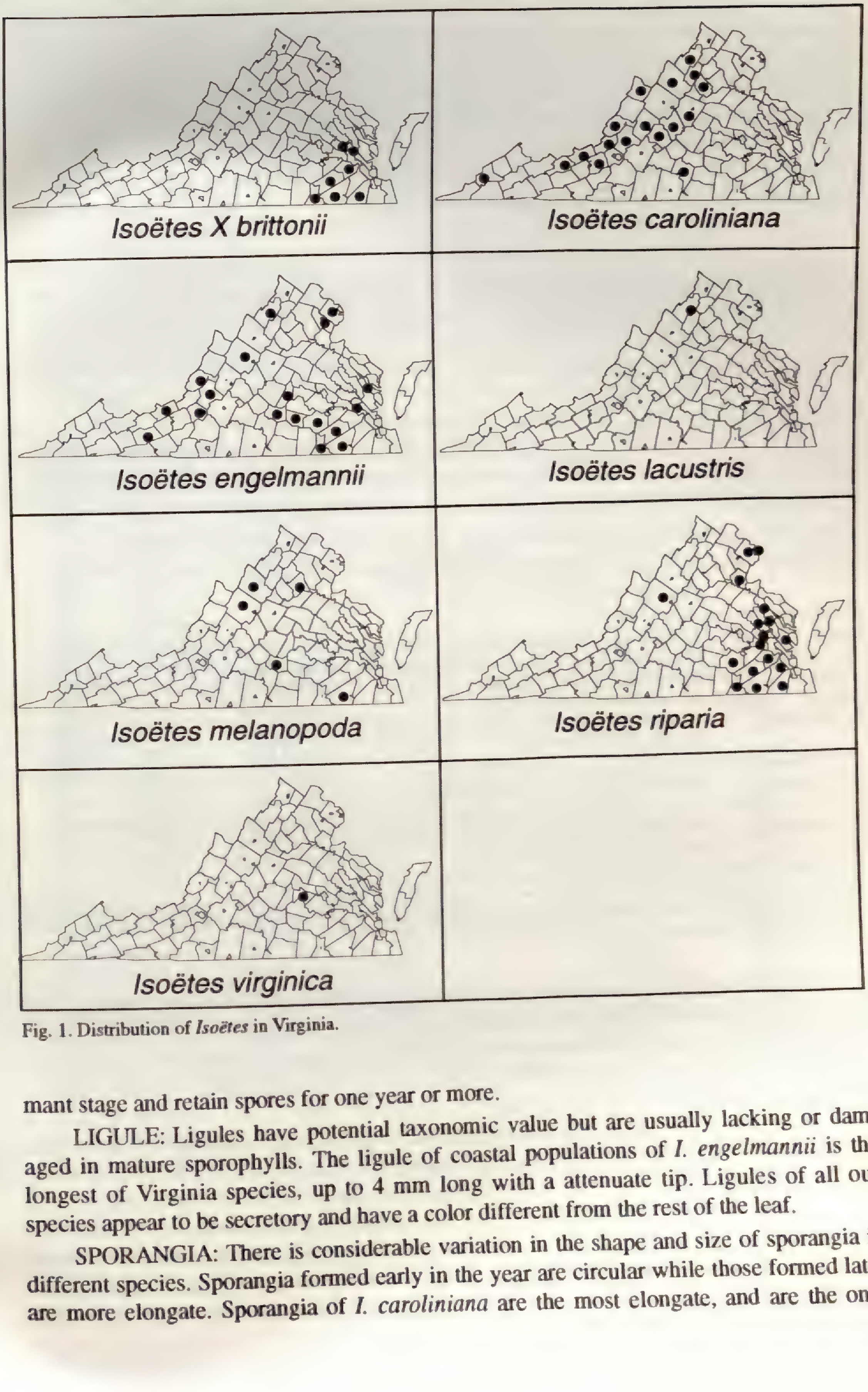

Fig. 1. Distribution of *Isoëtes* in Virginia.

mant stage and retain spores for one year or more.

LIGULE: Ligules have potential taxonomic value but are usually lacking or damaged in mature sporophylls. The ligule of coastal populations of *I. engelmannii* is the longest of Virginia species, up to 4 mm long with a attenuate tip. Ligules of all our species appear to be secretory and have a color different from the rest of the leaf.

SPORANGIA: There is considerable variation in the shape and size of sporangia in different species. Sporangia formed early in the year are circular while those formed later are more elongate. Sporangia of *I. caroliniana* are the most elongate, and are the only

sporangia which are consistently longer than broad. Pigmentation of the sporangial wall is helpful in separating taxa. *Isoëtes caroliniana* is the only one without a pigmented sporangial wall. All others are pigmented with flecks or streaks of brown or black although some mountain collections of *I. engelmannii* have clear walls.

VELUM: Aside from spore ornamentation, velum coverage is one of the few dependable features to be used in taxonomy of quillworts. Velum coverage is 33% or less in all species except *I. caroliniana* and *I. melanopoda* which may have 75% velum coverage. However, little is known about the variation of velum coverage within a taxon.

MEGASPORE SIZE AND SHAPE: Means and standard deviations of megaspores are provided for each taxon (Table 2). There is considerable overlap among most species. The shape of the megaspore is round in some species, eg, *I. engelmannii* and *I. caroliniana;* others are subglobose due to the fact that the proximal hemisphere is somewhat flattened, eg, *I. riparia, I. X brittonii.*

Table 2. Virginia *Isoëtes* megaspore measurements

Species	Collection(s)	Physiographic Province	Mean Size[1] (microns)	Standard Deviation
Isoëtes lacustris	Stevens, 15678 (FARM)	Mountains	692^{A}	24.2
Isoëtes riparia	Fernald & Long, 10081 (GH)	Coastal Plain	569^{B}	36.7
	Musselman & Knepper, 13009C (ODU)	Coastal Plain	540^{C,D}	32.2
	Musselman & Musselman, 13007C (ODU)	Coastal Plain	523^{D,E}	19.4
	Harvill, 16994 (FARM)	Coastal Plain	519^{D,E}	22.6
Isoëtes engelmannii	Wood, 6190 (US)	Mountains	494^{F,G,H}	11.8
Isoëtes X brittonii	Musselman & Knepper, 13009B (ODU)	Coastal Plain	499^{E,F,G,H}	30.4
	Musselman & Knepper, 13011B (ODU)	Coastal Plain	479^{H,I,J}	45.1
	Fernald & Long, 8913 (GH)	Coastal Plain	472^{H,I,J}	25.1
	Fernald & Long, 12915 (GH)	Coastal Plain	513^{E,F}	26.5
Isoëtes riparia	Svenson, 463 (FARM)	Coastal Plain	492^{F,G,H}	30.7
	Mikula, 2355 (FARM)	Coastal Plain	457^{J}	18.0
Isoëtes engelmannii	Musselman & Knepper, 13010 (ODU)	Coastal Plain	463^{I,J}	32.8
Isoëtes virginica	Musselman & Knepper, 13023 (ODU)	Piedmont	438^{K}	19.6
Isoëtes caroliniana	Wagner, 62218 (US)	Mountains	400^{L}	20.3
Isoëtes riparia	Wieboldt, 4732 (VPI)	Coastal Plain	359^{M}	22.1
	Musselman, 13004 (ODU)	Coastal Plain	322^{N}	21.0
Isoëtes melanopoda	Musselman, Aigbokhan, Stouffer, sn (ODU)	Piedmont	230^{O}	45.7

[1]Superscript denotes Student-Newman-Keuls range test grouping. Means with the same letter are not significantly different. N = 20 for each population.

The megaspores of *I. lacustris* are significantly larger (P=0.05) than those of any other Virginia quillwort. Although megaspore sizes of Virginia quillworts in this study are in general agreement with previously published megaspore size ranges (eg, Kott & Britton, 1983; Brunton & Taylor, 1990; Taylor, et al., 1993) topoclinal variation within a species may be obscured by computing "grand means." The SNK groupings in Table 2 indicate there is a general decrease in megaspore size within the same species from west to east in the state. For example, the megaspores of *I. engelmanii* collected from the mountains are significantly larger than megaspores collected from *I. engelmannii* of the Coastal Plain. A similar pattern exists for *I. riparia* of the outer coastal plain. Based on a cursory examination of the *I. riparia* collections, there does not appear to be a correlation between megaspore size and ornamentation. These differences in spore size may be a result of differing environmental conditions or potential differences in ploidy levels with-

in a species (Allison, personal communication).

EQUATORIAL RIDGE: This is the ridge which encircles the spore at approximately the middle. It may be unornamented, eg, *I. melanopoda,* slightly ornamented, eg, *I. X brittonii,* or flange-like with onamentation similar to that of the proximal hemisphere, eg, *I. engelmannii.* The ornamentation of the equatorial ridge should be distinguished from that of the girdle.

GIRDLE: The girdle is a band distal to the equatorial ridge which often has distinct ornamentation. Only two taxa, *I. X brittonii* and *I. riparia,* have well developed girdles. A very short girdle may be present in *I. caroliniana* and *I. engelmannii.*

PROXIMAL ORNAMENTATION: The proximal side of the spore has the tri-radiate crest. A point which needs emphasis in phytography of *Isoëtes* is the difference between the patterns of ornamentation on the distal and proximal hemispheres; this can vary among species (Hickey, 1986). *Isoëtes engelmannii* and to a lesser extent, *I. caroliniana,* have similar ornamentation on both the proximal and distal hemisphere, but in other taxa the ornamentation is often quite different. In general, the distal ornamentation is more uniform than the proximal. Near the top of the spore, the pattern of ornamentation may be somewhat obliterated.

DISTAL ORNAMENTATION: The distal side of the spore is the hemisphere opposite the tri-radiate ridge. Descriptions of distal ornamentation must distinguish between the girdle and the ornamentation of the rest of the distal hemisphere.

Table 3. *Isoëtes* microspore measurements

Species	Collection(s)	Physiographic Province	Mean Size[1] (microns)	Standard Deviation
Isoëtes lacustris	Wieboldt & Croy, 4229 (VPI)	Mountains	40[A]	1.9
Isoëtes melanopoda	Massey & Stevens, sn (GH)	Piedmont	34[B]	1.5
Isoëtes X brittonii	Musselman & Knepper, 13011B (ODU)	Coastal Plain	33[B]	1.6
Isoëtes virginica	Musselman & Knepper, 13023 (ODU)	Piedmont	31[C]	1.6
Isoëtes riparia	Musselman & Knepper, 13009C (ODU)	Coastal Plain	30[D]	1.8
	Musselman, 13004 (ODU)	Coastal Plain	30[D]	1.1
	Wieboldt, 4732 (VPI)	Coastal Plain	30[C,D]	1.3
	Fernald & Long, 10081 (GH)	Coastal Plain	31[C,D]	1.2
Isoëtes X brittonii	Fernald & Long, 12222B (GH)	Coastal Plain	30[C,D]	1.2
Isoëtes melanopoda	Musselman, Aigbokhan, Stouffer, sn (ODU)	Piedmont	29[E,F]	1.2
Isoëtes engelmannii	Wood, 6190 (US)	Mountains	28[E]	1.7
Isoëtes riparia	Harvill, 16994 (FARM)	Coastal Plain	26[F]	0.9
	Shull, 252 (GH)	Coastal Plain	25[F,G]	0.9
Isoëtes engelmannii	Fernald & Long, 10080B (US)	Coastal Plain	25[G]	1.3
Isoëtes caroliniana	Howard, Norris & Buchner, sn (US)	Mountains	21[H]	1.0

[1]Superscript denotes Student-Newman-Keuls range test grouping. Means with the same letter are not significantly different. N = 20 for each population.

MICROSPORE SIZE: Means and standard deviations are provided for each of the taxa. There appears to be less overlap among microspore size than megaspore size, which is in agreement with Kott & Britton (1983). Microspore measurements are summarized in Table 3. In comparison to megaspores, the microspores of individual quillwort species are relatively less variable in size and ornamentation (Kott & Britton, 1983). The statistical analysis of the microspore measurements in this study was identical to that for megaspores. Again, *I. lacustris* had the largest spores of any of the Virginia quillworts. Less

obvious was the intermediate nature of the hybrid quillwort spore in comparison to the putative parents and the higher degree of variability in spore size (marginally higher standard deviations). Like megaspores, there was a general trend in which the western collections of a given species had significantly larger microspores than those from the east.

MICROSCPORE COLOR: This refers to the color of the dry microspores *en masse* when shed from the microsporangium. *Isoëtes virginica* is distinct in having dark brown microspores. Other taxa are white, grey, or light tan.

MICROSPORE ORNAMENTATION: Microspore ornamentation, although tedious to use, is valuable in separating taxa. All Virginia quillworts have some form of ornamentation on the microspores except *I. lacustris.* Most populations of *I. engelmannii* have laevigate microspores; a few populations have tuberculate microspores. Descriptive terminology of microspores is less developed than those of megaspores. Although the equivalent terms of distal, proximal, and equatorial have been suggested by Berthet & Lecocq (1977), there are not suitable terms to describe the region where the spores were joined before being separated in meiosis. Like megaspores of hybrids, microspores are often variously shaped although the ornamentation does not vary as much as that of megaspores.

CHROMOSOME NUMBERS: These are taken from the literature (summarized in Taylor, et al., 1993; Kott & Britton, 1983) and from Bray & Chowdhary (personal communication).

SPORE MATURATION: Virginia quillworts mature at different seasons. The first to mature is *Isoëtes engelmannii* and the last is *I. caroliniana.* Dates are noted based on field work and herbarium studies and refer to megaspore maturation. In general, megaspores are produced earlier in the season than microspores as also noted by Mathews & Murdy (1969).

Key to Virginia Species of Isoëtes

Perhaps the old adage that "Keys are made by people who don't need them for people who can't use them" has no better application than with quillworts. In this key, we have tried to stress simplicity rather than providing an exhaustive key. We simply know too little about vegetative characters to put them in a key. Megaspores are essential for determination.

1. Megaspores of uneven size, shape and ornamentation with many broken and aborted spores.....1. *I. X brittonii* (other hybrids will key here)
   Megaspores of generally even size, shape and ornamentation .....2.

2. Megaspores more than 600 microns wide; submergent of mountain stream .....
   .....4. *I. lacustris.*

2. Megaspores less than 600 microns wide; submergent or emergents; diverse habitats
   .....3.

3. Equatorial ridge flared (wider than width of reticulum); girdle lacking or reduced (less than the width of a reticulum).....4.

3. Equatorial ridge not flared; girdle lacking or present .....5.

4. Surface honeycombed, ridges of homeycombs straight with knife-life edge; reticulations of approximately even dimensions; microspores laevigate to sparsely tuberculate; sporangial wall pigmented (mountain plants unpigmented).....3. *I. engelmannii.*
4. Surface of irregular reticulations, ridges sinuous of uneven height giving a jagged appearance; microspores spinulose, never laevigate; sporangial wall not pigmented. .....2. *I. caroliniana.*
5. Girdle lacking megaspores tuberculate or rugulate but never with isolated columns; phyllopodia often present; plants usually of rock outcrops .....6.

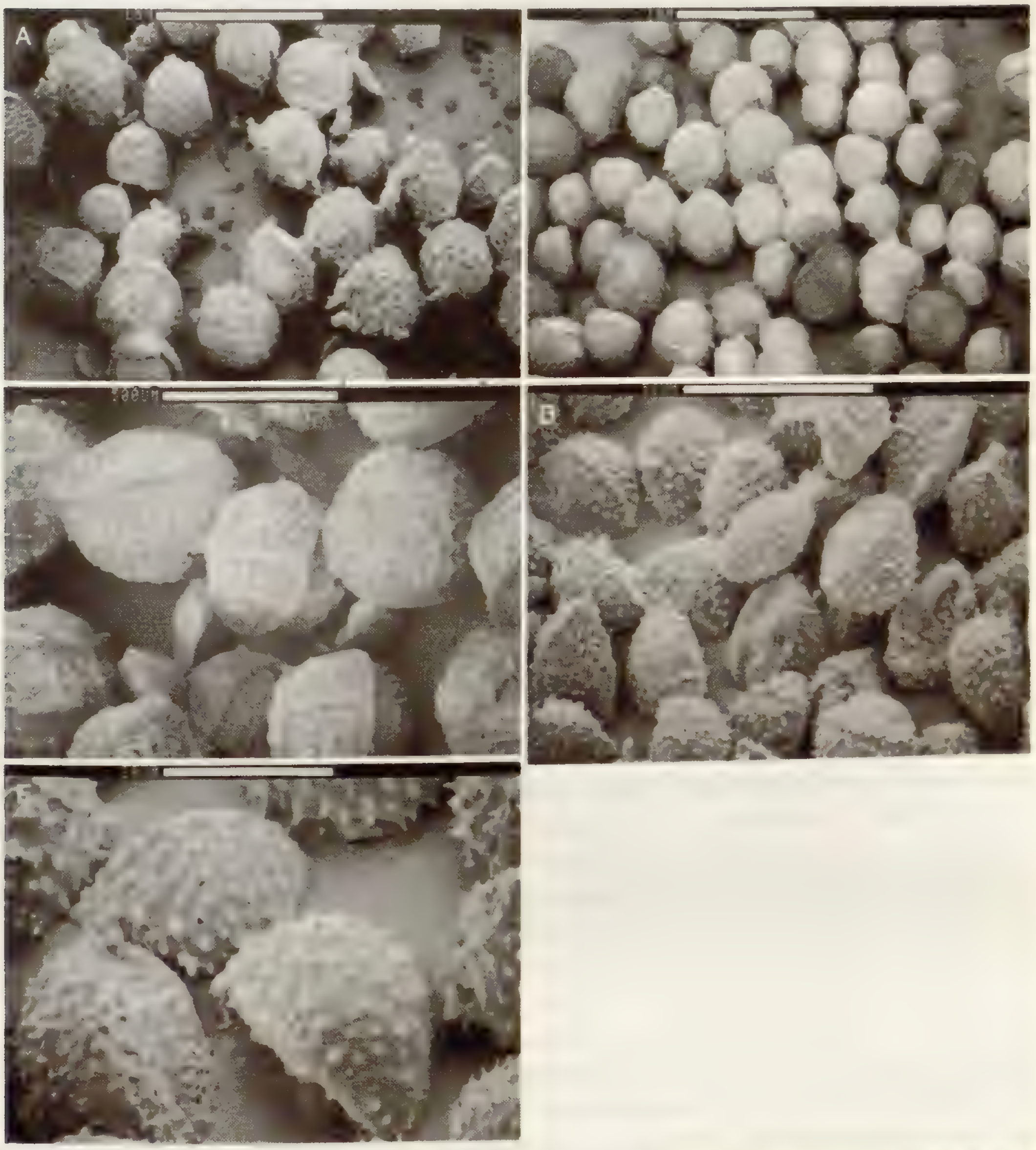

Fig. 2. *Isoëtes X brittonii.* A) General view of megaspores showing variation in size and morphology typical of hybrids. Most megaspores have an *"engelmannii"* type of ornamentation *(Musselman and Knepper, 139301).* Bar = 1 mm. B) General view of megaspores. Most megaspores have a *"riparia"* type of ornamentation. Bar = 1 mm. C) Detail of megaspores. Bar = 500 microns. D) Microspores. Bar = 50 microns. E) Microspores. Bar = 20 microns. (B, C, D, E all *Musselman and Musselman, 13006).*

5. Girdle well developed; plants of coastal streams and swamps; phyllopodia absent .....6. *I. riparia.*
6. Megaspores rugulate; microspores light tan .....5. *I. melanopoda.*
6. Megaspores tuberculate; microspores brown .....7. *I. virginica.*

Isoetes X brittonii Brunton and Taylor. Britton's Quillwort. HABITAT: Freshwater coastal streams and swamps. HABIT: Amphibious or aquatic. CORM: Round, seldom more than 2.5 cm in diameter. LEAVES: Typically few per plant (3–30), dull olive green to dark green, lax; to 45 cm long. SPORANGIA: Round or elongate; always pigmented. MEGASPORE SIZE AND SHAPE (Fig. 2A–C): 493 (418–550) microns; variable in shape. GIRDLE: Not ornamented to ornamented with crowded short columns. PROXIMAL AND DISTAL ORNAMENTATION: Uneven broken crests, rarely reticulate, with walls of uneven heights. MICROSPORE SIZE (Fig. 2D, E): 31.5 (28–36) microns. MICROSPORE ORNAMENTATION: Spinulose. SPORES MATURE: June to September. CHROMOSOME NUMBER: 2N=33 (Brunton & Taylor, 1990).

In the Coastal Plain of Virginia and adjacent North Carolina one of the most common quillworts and the most frequent hybrid is *Isoëtes X brittonii* which can be found wherever the two parents, *I. engelmannii* and *I. riparia* grow together. All three taxa are virtually identical vegetatively and can only be determined on spore characters. This hybrid has been reported (Brunton & Taylor, 1990) from Virginia [Fernald and Long, 13508 (NYS)], but the sheets we have from GH and US of this number are not *I. X brittonii.*

Isoëtes X brittonii is characteristically found along small, blackwater, primary streams. Water flow is heavy in the spring but later in the season these sites become considerably drier. Most of these quillworts are amphibious (as noted also by Fernald, 1937) and die back so that no trace of them is evident by late summer. Invariably, areas where road and/or bridge construction has taken place are suitable sites for *Isoëtes X brittonii.* The hybrid seems to be present in numbers more or less equal to those of the parent. It is not clear if the hybrid is continuously being formed or if there is some means of asexual reproduction. Ballou (unpublished) has germinated megaspores of *Isoëtes X brittonii* but it is not known if these are fertile.

Fernald (1937, 1940) collected *Isoëtes riparia* and *I. engelmannii* in the Nottoway River drainage of southeastern Virginia and another quillwort that he called "a most strange *Isoëtes*". The irrepressible Fernald notes: "Entangled with the *Hypoxis* was a most strange *Isoëtes* the exact identity of which I have been unable to make Mr. Weatherby divulge . . ." And in the same paper, Fernald goes on to say, "Another characteristic plant of the region, also found by us in June, 1936 and subsequently much collected, from May into July, on the rich wooded bottomlands, is a gigantic quillwort, *Isoëtes.* Flaccid and sprawling in the richest woods, with bulbs up to 3 cm in diameter and loose rosettes up to 9 dm across, it always facinates us, for it is decidedly not an aquatic; and on many bottomlands, from Dinwiddie County to Greensville and western Nansemond, it is impossible to walk in June without trampling upon it. It proves to be the little known *Isoëtes Engelmannii* var. *caroliniana* A. A. Eaton . . ." An examination of Fernald's collections indicate that this "strange *Isoëtes*" is often *Isoëtes X brittonii,* not *Isoëtes engelmannii* var. *caroliniana* now recognized as a distinct species (Luebke, 1992). Other specimens of the hybrids have been collected in the region but were not recognized as hybrids.

It is easy to understand how the usually precise Fernald could confuse this coastal plain hybrid with *I. engelmannii* var. *caroliniana* as there are similarities in megaspore ornamentation. Both taxa have irregular reticula and spinulose microspores. Boom (1980) noted that Fernald's "strange *Isoëtes*" may indeed be the hybrid between *I. engelmannii* and *I. riparia*. Our work confirms Boom's suggestion.

SPECIMENS EXAMINED. — DINWIDDIE: Wooded alluvial bottomland of Rowanta Creek (Variously spelled "Rowanta" and "Rowanty"), near Rowanta, *Fernald and Long, 8015C*, 8 June 1938, (GH); Sphagnous creek 4 mi. S of Rowanta, *Harvill, 31162*, 1 June 1974, (FARM); In Stony Creek at SR 666 crossing, *Musselman and Knepper, 13015, 13013B*, 25 June 1992, (ODU); Just N of SR 703 at Rowanty Creek, *Musselman and Knepper, 13013B*, 25 June 1992, (ODU); *Musselman and Musselman, 13007*, 12 June 1992; *Wieboldt, 6036*, 5 June 1986 (WM); South of SR 666 crossing of small, unnamed tributary of Stony Creek SE of 667 and 666 junction, *Musselman and Knepper, 13014*, 25 June 1992, (ODU); Wooded alluvial bottomland of Rowanta Creek, near Rowanta, *Fernald and Long, 8015*, 8 June 1938, (GH); Rich woods along Cat-tail Run, SE of Burgess Station, *Fernald and Long, 9797*, 27 May 1939, (GH); JAMES CITY: Bottomland woods of Powhatan Creek, NW of Five Forks, *Fernald and Long, 1321D*, 29 July 1941, (GH); GREENSVILLE: North banks of Fountain Creek, just W of US 301 bridge, *Musselman and Knepper, 13011B*, 17 June 1992, (ODU); Bare mud in cypress swamp along Fountain's Creek near SR 625 bridge, *Musselman and Knepper, 13017B*, 25 June 1992; Wooded bottomland of Fontaine Creek (Variously spelled as "Fontaine", "Fountain", and "Fountain's") SE of Taylor's Millpond, *Fernald and Long, 10079*, 12 and 13 June 1939, (GH); NANSEMOND: Wooded bottomland of Somerton Creek, about 2 mi. E of Factory Hill, *Fernald and Long, 8534*, 20 July 1938, (GH); Muddy tidal margin of Blackwater River, Milk Landing, S of South Quay, *Fernald and Long, 11217*, 22 September 1939, (GH); NEW KENT: Chickahominy River at SR 618 bridge, *Musselman and Musselman, 13025*, 27 June 1992, (ODU), Bank of Chickahominy River, SW of Providence Forge, *Harvill, 16994*, [sd], (FARM); SOUTHAMPTON: Muddy woodland brook about 2 mi N of Hugo, *Fernald and Long, 8913B*, 19 August 1938, (GH); Wooded bottomland, Three Creek, SW of Grizzard, *Fernald and Long, 10080A*, 14 June 1939. (US); Assamoosick Swamp at SR 35 bridge, SE of Sebrell, *Musselman and Knepper, 13002*, 1 May 1992, (ODU); North bank of Three Creek near Drewryville at SR 659 bridge, *Musselman and Knepper, 13009B*, 16 October 1992, (ODU); *Musselman and Knepper, sn*, 15 May 1991, (ODU); Sandy alluvial bottomland of Three Creek, Drewryville, *Fernald and Long, 5588A, C*, 22 June 1936, (GH): North of SR 635 bridge over branch of Seacock Swamp, *Musselman and Musselman 13006*, 12 June 1992, (ODU); SURRY: Bottomland woods along Blackwater River, about 1 mi SE of Dendron, *Fernald and Long, 12915B*, 14 June 1941, (GH); SUSSEX: Wooded bottomland, Jones Hole Swamp, W of Coddyshore, *Fernald and Long, 10081*, 16 June 1939, (GH); Woods by Nottoway River, SE of Lamb's, *Fernald and Long, 12222B*, 18 July 1940, (GH).

2. *Isoëtes caroliniana* (A. A. Eaton) N. T. Luebke. Carolina Quillwort. HABITAT: Streams, seepage areas, ponds, impoundments, and lakes. HABIT: Aquatic or amphibious. CORM: Round, up to 5 cm in diameter. LEAVES: Yellow green, to 35 cm, lax; evergreen. SPORANGIA: Elongate; no pigmentation. VELUM: Covering more than 33% of sporangium. MEGASPORE SIZE AND SHAPE (Fig. 3A and B): 400 microns (374–440); round. EQUATORIAL RIDGE: Well developed, wider than a reticulum. GIRDLE: Very slight. PROXIMAL AND DISTAL ORNAMENTATION: Reticulate but with 50% or less of the reticula not enclosed; walls of uneven heights and widths. MICROSPORE SIZE: 21 microns (19–22). MICROSPORE ORNAMENTATION (Fig. 3C and D): Shallowly spinulose. CHROMOSOME NUMBER: 2N=22 (Bray & Chowdhary, personal communication; Taylor, et al. 1993). This report (Bray and Chowdhary, personal communication) is apparently the first for this species. Wagner & Wagner (1966) report a count of N=11 for *I. engelmanii* from Giles County. This is probably *I. caroliniana* as all quillworts we have seen from Giles County are *I. caroliniana*. Taylor (1992) cites Wagner & Wagner (1966) as the reference for the chromosome number of *I. engelmanii*. SPORES MATURE: August to October.

Isoëtes caroliniana is an Appalachian endemic found in North Carolina, Tennessee,

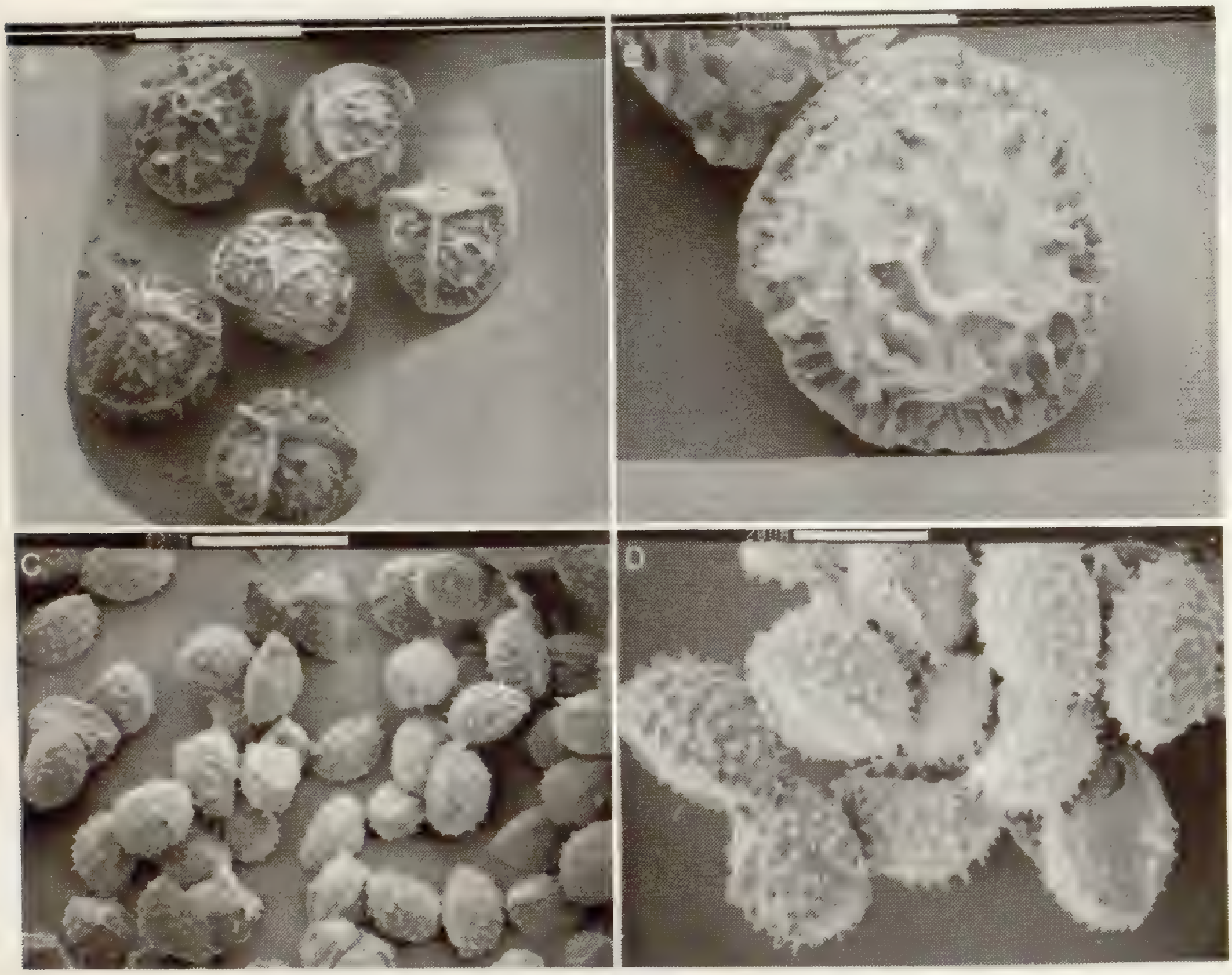

Fig. 3. *Isoëtes caroliniana*. A) General view of megaspores *(Wagner, 62218)*. Bar = 500 microns. B) Distal surface of megaspore *(Canby, sn)*. Bar = 200 microns. C) Microspores *(Musselman and Knepper, 13051)*. Bar = 50 microns. D) Detail of microspores *(Harvill, 14586)*. Bar = 20 microns.

and Virginia (Luebke, 1992) and should be looked for in the mountains of contiguous states. It was formerly treated as a variety of *I. engelmannii*. Luebke (1992) elevated it to specific status. For many years it was studied in the mountains as *I. engelmannii* (eg, Radford, 1950; Wagner & Wagner, 1966) but is readily distinguished from that taxon by the usually unpigmented sporangial wall (eg, Parker, 1943).

In general, *I. caroliniana* is more robust than *I. engelmannii* with larger leaves, a greater number of leaves, and a large, flat corm. It is our largest quillwort. Duff & Evans (1992) and Duff & Schilling (1993) give further evidence for the specific status of *I. caroliniana* based on allozyme and cpDNA work. They suggest that *I. caroliniana* may be derived from the widespread *I. engelmannii*. Because of this affinity and sympatry, hybrids between these two should be searched for. Some of the specimens included in our study may, in fact, be hybrids between these two species.

Specimens Examined — ALLEGHANY: Edge of Craig County, dried pond, *Harvill, sn*, 23 July 1966, (TENN); ALBERMARLE: Ivy Creek headquaters, *Stevens, 106*, 3 October 1966 (NA); *Stevens, 11010*, 6 July 1975, (FARM); AMHERST: Brook edge 2 mi. E of Blue Ridge parkway, SR 607, *Harvill, 22473*, 14 August 1969, (FARM); BLAND: Kimberling Creek near Giles county line, *Musselman and Musselman, 13060*, 16 August 1992, (ODU); BOTETOURT: Craig Creek, 1.5 mi. S of Oriskany, *Wieboldt, 4188*, 1 August 1981, (VPI); Craig Creek at SR 710 ford, *Musselman, sn*, 25 September 1993 (ODU); CHARLOTTE: Seepage area 0.5 mi. W of SR 47, N of Vincent Corner, *Musselman, 13910*, 4 July 1993, *Knepper and Musselman, 139311*, 22 July 1993 (ODU); CRAIG: In wet area on top of Kelly Knob, *Massey, 415*, 16 October 1941, (VPI);

Temporary Pond, Big Pond, *Harvill, 14586,* 26 July 1966 (FARM); FLOYD: 1 mi. N of Mabry Mill, Blue Ridge Parkway, *Mikula, 6150,* 7 July 1950 (WM); GILES: Mountain Lake, Salt Pond Mountain, *Canby, sn,* August 1890; (US), *Lewis, sn,* 20 July 1930, (VPI); *Oakes, sn,* 28 August 1936, (VPI); *Given, sn,* 1937, (VPI); Mountain Lake Biological Station of the University of Virginia, *Howard, Norris, and Buchner, sn,* 19 November 1977, (US); *Laursen, sn,* 12 October 1972, (FARM); *Laursen, 066, 068,* 10 December 1972, (VPI); *Moser, sn,* 13 October 1974, (VPI); *O'Donnell, 77,* 30 June 1987 (VPI); *Musselman and Knepper, sn,* 14 May 1990, (ODU); *Gregg and Wilson, sn,* 19 October 1977, (VPI); Warden's Trail near top of Flat Top Mt. *Massey, 3981,* 7 August 1940, (VPI); Road to Cascades from house at Little Meadows, *Wagner, 62218,* 5 August 1962, (US); *Wagner, 62210,* 5 August 1962, (NA, TENN, VPI); Kelly's Flats along Big Stony Creek, *Evans, sn,* 1 July 1980, (TENN); Flat Top Mountain, Warden's Spring, *Wood, 590,* [sd] 1940, (VPI); John's Creek Valley, *West, 8679* (LYNCH); HIGHLAND: Just S of SR 642 near West Virginia state line, *Stevens, 12650,* 5 June 1976, (FARM); Beaver pond at head of Buck Run, *Stevens, 18277,* 21 August 1982, (FARM); MADISON: Big Meadows Swamp, *Fosberg, 34911,* 18 August 1953, (US); *Stevens and Harvill, 20234,* 31 August 1968, (FARM); *Harvill and Lehman, 15412,* 23 October 1966, (NA, US); *Mazzeo, 2145,* 4 July 1967, (NA); *Musselman and Knepper, 130513,* 5 August 1992, (ODU); Muddy stream, 0.3 mi. SE of Big Meadows Campground, *Fleming, 5260,* 17 September 1990, (GM); MONTGOMERY: Pandapas Pond, *Porter, 202,* 23 September 1971, (VPI); NELSON: Wintergreen Resort, *Coleman, 45329,* 30 June, 1991, (LYNCH); PAGE: Headwaters of Passage Creek, *Fessenden, sn,* 21 October 1951, (US); Hawksbill Mountain, *Mikula, 5406,* 21 June 1950, (FARM); ROCKBRIDGE: Shallow margins of Maury River, 6 mi. N of Goshen Pass, *Musselman and Musselman, sn,* 21 June 1993 (ODU); ROCKINGHAM: 0.25 mi NE of Hawksbill Creek, *Stevens, 11498,* 3 August 1975, (FARM); SHENANDOAH: Great Swamp in Massanutten Mts., *Fessenden, sn,* 30 May 1941; Peters Run, *Harvill, 21727,* 29 June 1969, (FARM); Mudhole bog, Little Powells Fort, *Artz, 858,* 3 July 1948, (GH); Jake Funk's spring, *Artz, 367,* 21 July 1953, (VPI) Peter's Mill Run Bog, Little Fort, *Artz, sn,* 7 July 1966, (VPI); WISE: Headwaters of Burns Creek, *Clampitt, 1340,* 7 June 1993 and *Clampitt and Belden 1464,* 22 July 1993 (ODU).

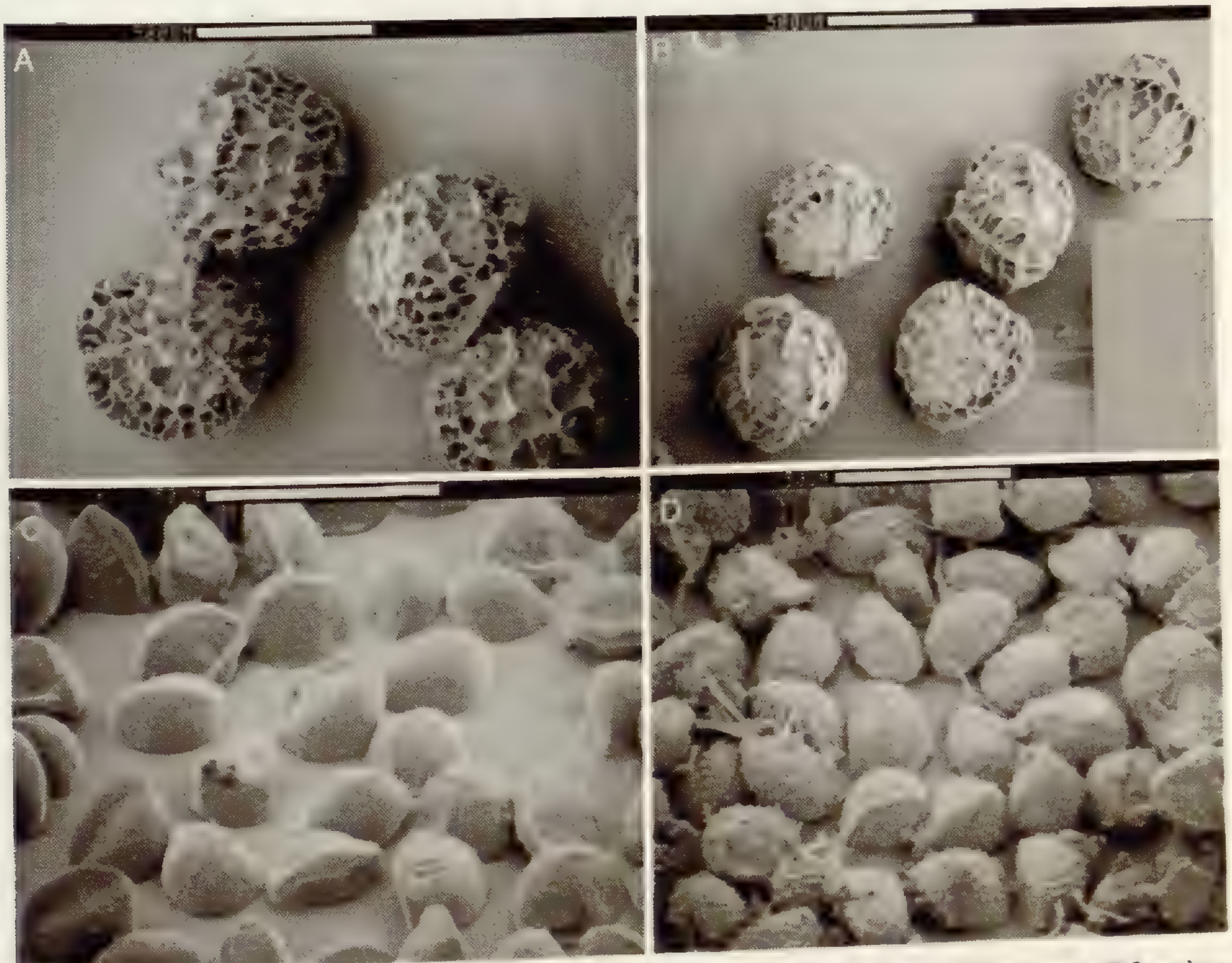

Fig. 4. *Isoëtes engelmannii.* A-B) Megaspores *(Mikula, 1774).* B) *Wood, 6190.* Bars = 500 microns. C) Laevigate microspores D) Tuberculate microspores. (C&D) *Musselman and Knepper, 13003.* Bars = 50 microns.

3. *Isoëtes engelmannii* A. Braun. Engelmann's Quillwort. HABITAT: Freshwater streams, lakes, ponds, and swamps. HABIT: Aquatic or amphibious. CORM: Two lobed, conical, seldom more than 4 cm wide. LEAVES: Up to 60 cm. Not evergreen when amphibious. LIGULE: Up to 4 mm, attenuate. SPORANGIA: Round to long oval; wall brown streaked, clear in some mountain populations. VELUM: Covering 33% of sporangium. MEGASPORE SIZE (Fig. 4A and B): Mountain 494 (484–517) microns; Coastal Plain 463 (396–506) microns. EQUATORIAL RIDGE: Flared, wider than a single reticulum (Fig. 4A). GIRDLE: Slightly ornamented with broken columns. Girdle narrower than a single reticulum. PROXIMAL AND DISTAL ORNAMENTATION (Fig. 4A and B): Honeycombed with most reticula enclosed on all sides. Walls of reticula with knife edges and or + - equal height. Similar in the two hemispheres although the promixal ornamentation may not form reticula near the crest of the spore. MICROSPORE SIZE (Fig. 4C and D): Mountains 28 (25–32); Coastal Plain 25 (22–26). MICROSPORE ORNAMENTATION: Laevigate (Fig. 4C) to slightly tuberculate (Fig. 4D). CHROMOSOME NUMBER: 2N=22 (Kott & Britton, 1983). SPORES MATURE: June to frost (if aquatic) or June to late summer if amphibious.

Isoëtes engelmannii is the most widespread quillwort in Virginia and Eastern North America. It is found in a diversity of habitats throughout the state. In the Coastal Plain, it is vegetatively identical to other taxa.

SPECIMENS EXAMINED. — AUGUSTA: Kennedy Mt, *Carr, sn, sd,* (VPI); BOTETOURT: Open bedrock area of Craig Creek. 1.3 mi NW of Oriskany, *Wieboldt, 4235,* 25 September 1981, (WM, VPI); Along Craig Creek, 1.3 mi S of Oriskany, *Musselman and Musselman, 13056,* 16 August 1992; Along Craig Creek, 1.5 mi S of Oriskany, *Musselman and Musselman, 13055,* 16 August 1992 (ODU); CUMBERLAND: In Bear Lake, *Seay, sn,* 6 July 1960, (VPI); DINWIDDIE: Wooded alluvial bottomland of Rowanta Creek near Rowanta, *Fernald and Long, 8015D,* 8 June 1938, (GH); Cattail Run near Burgess Station, *Fernald and Long, 9797,* 21 May 1939, (FARM, US); In unnamed tributary of Stony Creek at SR 666, SE of 667, *Musselman and Knepper, 13014B,* 25 June 1992, FAIRFAX: Rock outcrop and pasture along Elklick Run, *Bradley, Fredericksen, and Deeb, 10442,* 23 May 1976, (GM); GREENSVILLE: Fountain Creek at 603 Bridge, *Musselman and Knepper, 13010,* 17 June 1992, (ODU); Fountain Creek at 675 Bridge, *Musselman and Knepper, 13013A,* 24 June 1992, (ODU); North banks of Fountain Creek, just W of US 301 bridge, *Musselman and Knepper, 13011A,* 17 June 1992, *Musselman and Knepper, 13005,* 17 June 1992, (ODU); Otterdam Swamp on Greensville-Sussex Co. line, *Harvill, 21561,* 14 June 1969, (FARM); JAMES CITY: About 2 mi SE of Chickahominy Bridge, *Mikula, 4845,* 14 June 1950, (FARM); Bottomland woods along Powhatan Creek, *Fernald and Long, 12915,* 29 July 1941, (GH); MIDDLESEX: Along Piankatank River, 2 mi N of Glenns Forks, *Leonard and Killip, 34,* 29 May 1921, (GH, US); Dragon Run just SE of SR 603 bridge, *Knepper and Musselman, 13003,* 20 May 1992, (ODU); NOTTOWAY: 2 mi SE of Gainsville [Spainville] in sluggish brook, *Wherry, sn,* 30 May 1934, [VPI (two sheets), NA]; 3 mi E of Gainsville [Spainville], *Benedict, 2778,* 30 May 1934, (VPI); PRINCE EDWARD: Goodwin Lake, Prince Edward State Park, *Dixon, sn,* 17 July 1972, (FARM); PRINCE WILLIAM: 1 mi E of Gainsville, *Spawn, 89,* June 1938, (VPI); ROANOKE: Spring marsh, 3.5 mi WSW of Salem, *Wood, 6190,* 12 July 1946, (GH, US); Bennett Springs, 3 mi N of Hanging Rock, *Wood, 6199,* 12 July 1946 (GH); SHENANDOAH: sd., *Handley, 298,* (VPI); Passage Creek at Elizabeth Furnace, *Wieboldt and Croy, 4229A,* 28 August 1981, (WM); SOUTHHAMPTON: Nottoway River at end of SR 742, Courtland, *Musselman and Madden, 13904,* 27 May 1993 (ODU); Muddy woodland brook about 2 mi N of Hugo, *Fernald and Long, 8913A,* 19 August 1938, (GH); Wooded bottomland, Three Creek, SW of Grizzard, *Fernald and Long, 10080,* (GH), *10080B,* (US), 14 June 1939; Assamoosick Swamp at SR 35 bridge, SE of Sebrell, *Musselman and Knepper, 13002* (ODU); Sandy alluvial bottomlands of Three Creek, Drewryville, *Fernald, Long, and Smart, 5588B,* 22 and 23 June 1936, (US); North bank of Three Creek near Drewryville at 659 bridge, *Musselman and Knepper, 13009A,* 16 October 1992, (ODU); North of 635 bridge over branch of Seacock Swamp, *Musselman and Musselman 13006A,* 12 June 1992, (ODU); Wooded bottomland of Nottoway River, Cypress Bridge, *Fernald and Long, 597A,* 25 July 1936, (GH, US); Sandy tidal margin of Nottoway River, Battle Beach, *Fernald and Long, 10082,* 23 June 1939 (GH); SUSSEX: Assamoosic Swamp, 3 mi E of Homeville, *Mikula, 1774,* 24 June 1949, (FARM); Wooded bot-

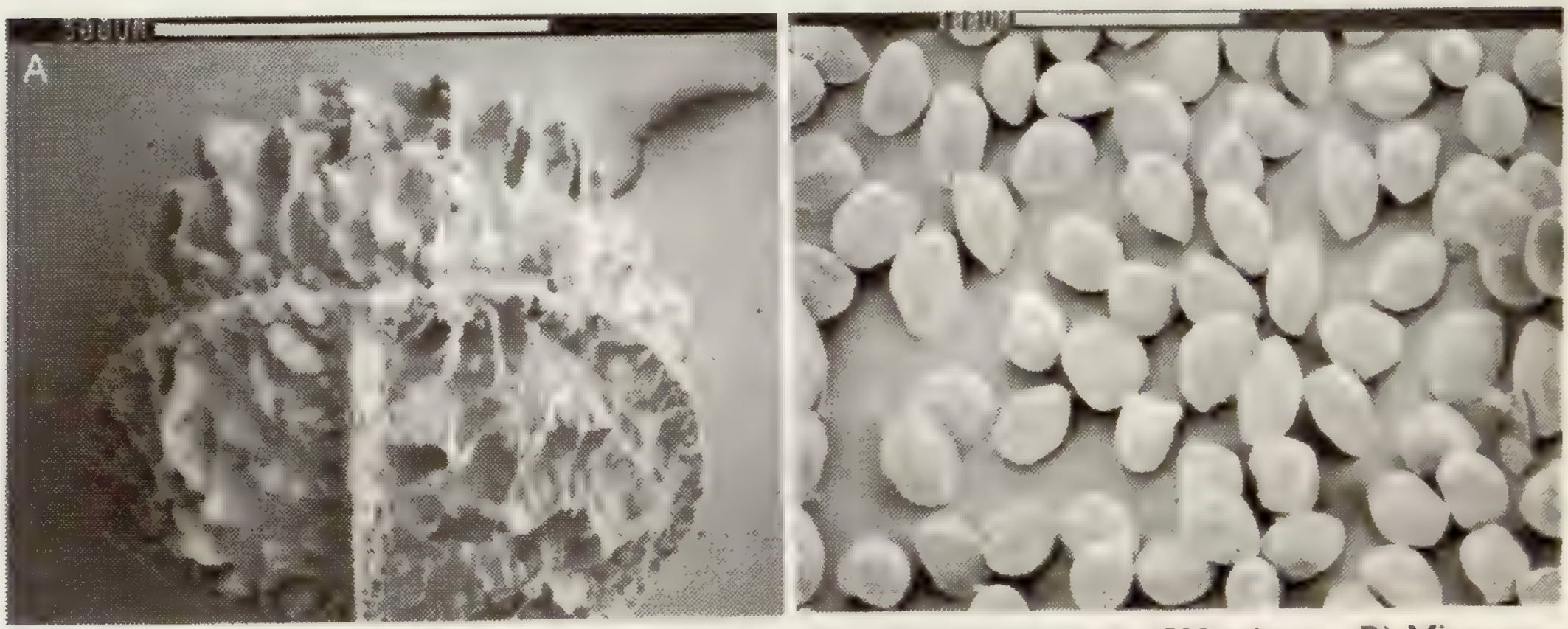

Fig. 5. *Isoëtes lacustris*. A) Megaspore. *(Griscom and Svenson, 5561.)* Bar = 500 microns. B) Microspores *(Wieboldt, 4229)*. Bar = 100 microns.

tomland, Three Creek, SW of Grizzard, *Fernald and Long, 10080*, 14 June 1939, (GH, US); SR 653 bridge, 5.2 mi NE of Meherrin River, *Ware and Roe, 6298*, 28 May 1975, (WM); WYTHE: Along Reed Creek, 0.1 mi SW of Cove Creek, 2.7 mi W of Max Meadows, *Wieboldt, 5660*, 27 June 1985, (WM).

4. *Isoëtes lacustris* L. Lake Quillwort. HABITAT: Cold mountain stream. HABIT: Aquatic. CORM: Globose. LEAVES: Dark green, to 14 cm, stiff; evergreen. SPORANGIA: Round; pigmented. VELUM: Covers 33% of the sporangium. MEGASPORE SIZE AND SHAPE (Fig. 5A): 692 microns (638–726); spherical. PROXIMAL ORNAMENTATION: Undulating ridges of uneven heights, becoming shorter near top of spore. DISTAL ORNAMENTATION: Irregular, thick reticulations with rounded tops. MICROSPORE SIZE AND SHAPE (Fig. 5B): 40 (35–44) microns. MICROSPORE ORNAMENTATION: Psilate. SPORES MATURE: July-August. CHROMOSOME NUMBER: 2N=110 (Kott & Britton, 1983).

Isoëtes lacustris (=*I. macrospora* Durieu) is a wide ranging, boreal species (Kott & Britton, 1983) which reaches its southern limit in Tennessee (Dennis, et al., 1979) with a single station in Virginia. The Virginia site is well known to botanists and is in danger of extirpation due to heavy recreational use (the population is in a stream in a popular campground) and, to a lesser extent, from overcollecting. A further threat is extinction by hybridization as noted for other *Isoëtes* by Allison (1992). *Isoëtes lacustris* is endangered in Virginia (Wagner & Taylor, 1991).

The Virginia population differs considerably from descriptions of the plant in other parts of its range. According to Kott & Britton (1983), *I. lacustris* has megaspores with a "wide band of large, individual papillae" on the distal side of the equatorial ridge. No girdle is present on the megaspores of the Virginia plants. The size of the megaspores is consistent with other reports of *I. lacustris*. However, the microspores of *I. lacustris* have been described as papillate (Kott & Britton, 1983; Taylor, et al., 1993) to spinulose (Pfeiffer, 1922), while collections from Virginia have smooth microspores. Boom (1979, 1982) and Fernald (1950) also described the southern disjunct populations of *I. lacustris* as having smooth microspores.

The hybrids at the Passage Creek site have not been previously noted although several sheets have hybrids. These include: *Musselman and Knepper, 13050* (ODU); *Stevens, 15678*, 14 July 1979, (VPI, FARM); *Evans, sn*, 8 November 1978, (TENN).

Taylor (1992) reported a hybrid between *I. lacustris* and *I. engelmannii* in New

Jersey, known from a single locality. The size of the spores strongly suggest that *I. lacustris* is one of the parents. We predict that the other parent is *I. engelmannii* based on the ornamentation of the microspore, relative megaspore sizes as well as its presence in this population [*Wieboldt and Croy, 4229A,* (WM but not VPI)].

Three of the four earlier gatherings from Passage Creek (in 1933, 1950, and 1981) are entirely *I. lacustris.* The 1978 collection *(Evans)* and the 1979 collection *(Stevens)* are hybrids. This suggests that the hybrid could be displacing the original population of *I. lacustris.* A similar situation has been reported from granite outcrop species (Allison, 1992).

SPECIMENS EXAMINED. — SHENANDOAH: Passage Creek at Elizabeth Furnace, *Brunton and McIntosh, 11, 169,* 6 July, 1992 (OAC), *Griscom and Svenson, 5561,* 2 July 1933, (GH); *Mikula, 5120,* 29 June 1950, (FARM); *Stevens, 15678,* 14 July 1979, (VPI, FARM); *Wieboldt, 4229,* 28 August 1981, (VPI, WM).

5. *Isoëtes melanopoda* Gay & Durieu. Blackfooted Quillwort. HABITAT: Rock outcrops (one collection from riverbottom forest). HABIT: Amphibious. CORM: Round, up to 3 cm in diameter. LEAVES: Bright green, stiff, up to 45 cm; phyllopodia present in all our specimens. SPORANGIA: Round (rarely elongate); pigmented. VELUM: Covering up to 75% of the sporangium. MEGASPORE SIZE AND SHAPE (Fig. 6A and B): 310 (237–405) microns; round. PROXIMAL ORNAMENTATION: Tuberculate. DISTAL ORNAMENTATION: Shallowly rugulate. MICROSPORE SIZE (Fig. 6C and D): 31 (29–35) microns. MICROSPORE ORNAMENTATION: Spinulose. SPORES MATURE: July to September. CHROMOSOME NUMBER: 2N=22 (Taylor, et al., 1993).

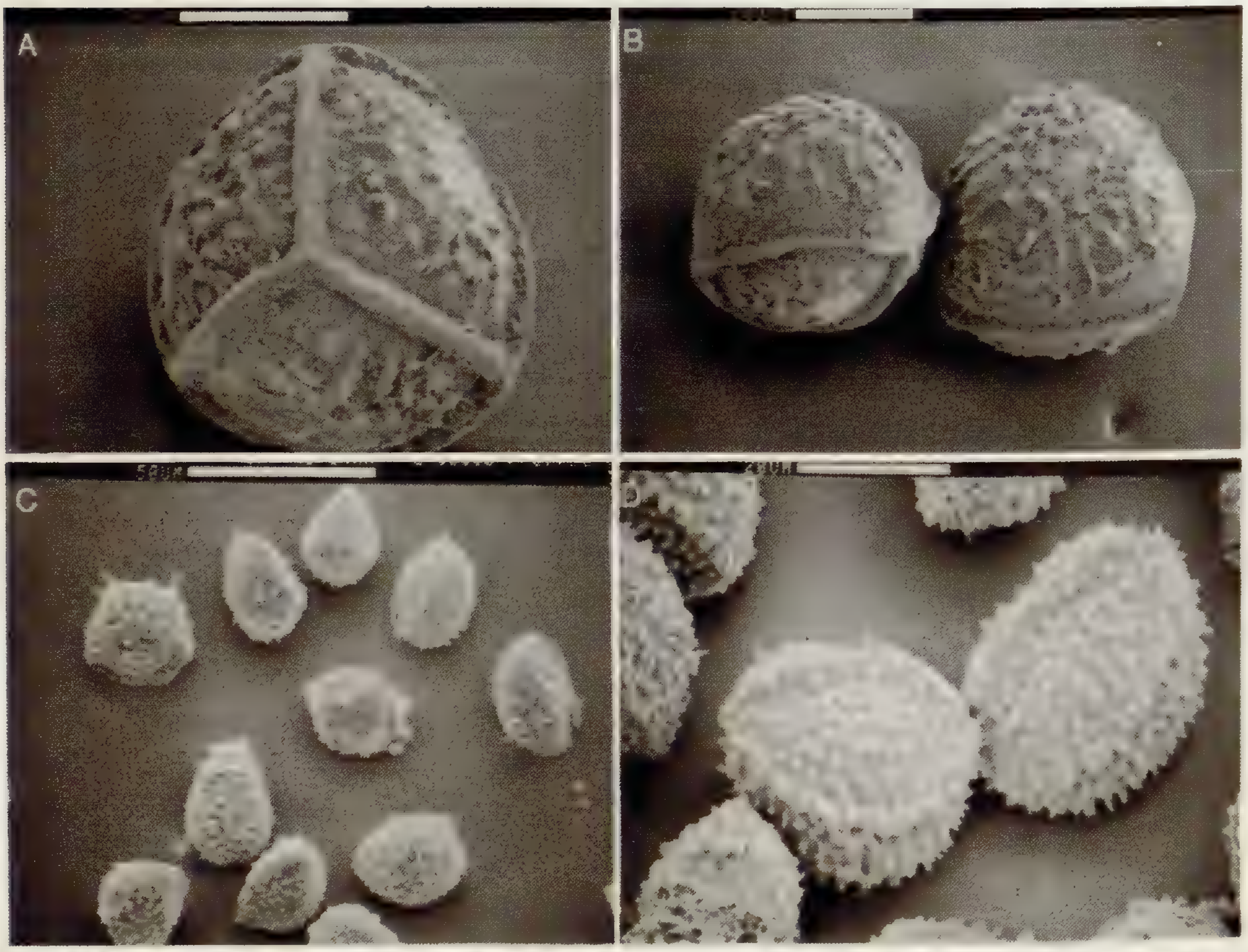

Fig. 6. *Isoëtes melanopoda.* A) Proximal surface of megaspore. *(Fernald et al., 5589).* B) Distal surface of megaspores *(Carr, sn).* Bars = 200 microns. D) Mircospores *(Musselman and Musselman, 13901).* Bar = 50 mircons. E) Microspores *(Massey, sd, 13 August 1938).* Bar = 20 microns.

Throughout most of its range *I. melanopoda* is usually found on non-calcareous rocks (Taylor et al., 1993), but in Virginia it has been collected in diverse habitats. Most unusual is the collection by Fernald, et al. along the Nottoway River. Despite extensive field work in this part of Virginia, we have never found *I. melanopoda* in a bottomland alluvium. Fernald was a fastidious collector and it is unlikely that his specimens were confused with other collections especially as he did very little collecting in Virginia in regions where *I. melanopoda* could be expected. Fernald described the *I. melanopoda* he collected (*Fernald, Long, and Smart, 5589)* when he writes "The alluvium of Nottoway River at Courtland was briefly visited. The strange *Isoëtes* was also there making solid turf in otherwise plantless depressions" (Fernald, 1937).

We have studied populations of *I. melanopoda* from Kentucky, North Carolina, and Virginia and have found remarkable variation in the size of the megaspores although the ornamentation is uniform even on the smallest spores.

Specimens Examined. — AUGUSTA: Pond vic. Stuart's Draft, *Carr, 458*, 4 July 1937, (GH, VPI); Near Moreheads, vic. Stuart's Draft, *Carr, 140*, 4 June 1936 (VPI); Dry ponds on Kennedy Mt., 3.5 mi S of Stuart's Draft, *Wherry, sn*, 16 July 1936 (TYPE of *I. virginica*, in part), (VPI); CULPEPER: In shallow water of puddle in triassic sandstone flatwoods ca. 1 mi ENE of Lagrange, *Stevens, 19271*, 26 May 1985, (FARM); Shallow water over flatrocks along 610 just W of junction with 609 (Lagrange), *Musselman and Musselman, 13008*, 13 June 1992, (ODU); Roadside ditch, 1 mi W of SR 609, *Musselman, Aigbokhan, Stouffer, sn*, 20 February 1993, (ODU); PRINCE EDWARD: In small drainage ditch in open woodland 1.5 mi NW of Farmville, *Massey and Stevens, sn*, 13 May 1938, (GH, VPI); ROCKINGHAM: Peaty soil of small acid summer dry pond 2.5 miles E

Fig. 7. *Isoëtes riparia.* A) Proximal and distal views of megaspores. *(Fernald, 10081).* B-C) *"Tidal" form of I. riparia.* C) *(Shull, 252).* Bars = 500 microns. D) Microspores *(Muselman, 13004).*

of Grottoes, *Stevens, 163,* 29 June 1967, (FARM); SOUTHAMPTON: Argillaceous and siliceous alluvium bottomland of Nottoway River, near Courtland, *Fernald, Long, and Smart, 5589,* 23 June 1936, (GH).

6. *Isoëtes riparia* Engelmann. Shore Quillwort. HABITAT: Freshwater and brackish water. HABIT: Aquatic or amphibious. CORM: Strongly two lobed, seldom more than 3 cm in diameter. LEAVES: 10–15 per plant (freshwater), 10–30 (brackish); to 50 cm in freshwater, seldom more than 40 cm in brackish; often recurved in brackish water; lax and sprawling in freshwater; evergreen in brackish water, non evergreen in freshwater. SPORANGIA: Round. MEGASPORE SIZE (Fig. 7A-C): Means 322–569 (range 279–594) microns. GIRDLE: Prominent in freshwater populations; poorly developed in brackish populations. PROXIMAL AND DISTAL ORNAMENTATION: Isolated columns or short, broken ridges (freshwater); slightly to moderately tuberculate (brackish). MICROSPORE SIZE: Means 26–30 (range 24–34) microns. MICROSPORE ORNAMENTATION: Spinulose (Fig. 7D). CHROMOSOME NUMBER: 2N=44 (Kott & Britton, 1983). SPORES MATURE: June and July.

Isoëtes riparia is our most variable quillwort. Except for gatherings in Augusta County, it is restricted to the Coastal Plain where it is found in two distinct habitats. The first is in tidal marshes where it is part of guild of plants including *Peltandra virginica, Pontederia cordata, Lilaeopsis chinensis, Eriocaulon parkeri,* and *Sagittaria subulata.* Here it often forms dense mats with numerous of individuals in a small area. The second habitat is that of small streams or non-tidal regions of coastal rivers. At these sites, it is either amphibious or, less frequently, aquatic. In swamp forests it may grow solitary in mud at the base of *Taxodium distichum* and *Nyssa aquatica* trees. More frequently, it grows near the waterline of small, blackwater streams with *Callitriche heterophylla, Micranthemum umbrosum,*and especially *Murdannia (=Aneilema) keisak,* an introduced weed (Dunn & Sharitz, 1990), which appears to be outcompeting *I. riparia* at many sites.

The morphology of the freshwater and tidal plants can be strikingly different. In general, the brackish plants have much shorter leaves seldom more than 10 cm long with recurved tips while the freshwater plants have long, lax leaves which spread on the surface of the ground. When both morphotypes are grown under the same conditions they retain their distinct growth forms. Variation within this species is well known and has been discussed in some detail by Proctor (1949) and Kott & Britton (1983). Various varieties have been described but further studies are necessary to determine the taxonomic status of the two forms. There is considerable range in the size of the megaspores (Table 2) with statistically significant differences among several populations (P = 0.05) but no correlation between freshwater and brackish habitats and spore size. Megaspore ornamentation is also variable. The brackish plants generally are tuberculate with few isolated columns (Fig. 7B–C) while the freshwater plants have distinct columns and are not tuberculate (Fig. 7A). The Mecklenburg County population *(Knepper and Musselman, 139401)* with 2N = 44 (Bray and Caplen, unpublished) has differing spore ornamentation and deserves further study.

Isoëtes riparia was once common in tidal rivers, both brackish and fresh. The descriptions of Fernald are valuable in documenting the decline of *I. riparia.* In reference to the Nottoway River, Fernald (1937) notes: " . . . our great prize here was the first collection of the season (by no means the last) of the estuarine *Isoëtes saccharata* not seen by Pfeiffer from south of the Potomac at Alexandria. Later in the summer it proved to be a regular inhabitant of tidal shores." Later, Fernald (1940) writes regarding collecting

along the James River. "The tide was rapidly making but outside the dense swale we were able to get the inevitable *Isoëtes saccharata.*" Unfortunately, *I. riparia* has been extirpated from much of Chesapeake Bay, a situation noted over 40 years ago by Proctor (1949). Taylor (1992) discusses a similar decline in New Jersey attributed to lower water quality. We have found very few plants on the lower Nottoway River where Fernald noted it being abundant.

SPECIMENS EXAMINED. — ARLINGTON: SW of Alexandria, *Shull, 201,* 16 October 1902, (GH): *Shull, 252,* 22 August 1902, (US); Fourmile Run, near Alexandria, Virginia, *Tidestrom, sn,* 12 July 1905, (NA, GH); AUGUSTA: Shallow water of creek near Spring Pond, *Harvill, Freer, and Harvill, 17232* (LYNCH); Between Sherando and Stuart's Draft, *Frank and Croy, 415,* 23 July 1980, (VPI); CHARLES CITY: Sandy tidal shore of James River below Harrison Point, *Fernald and Long, 11216,* 16 September 1939, (GH); DINWIDDIE: Wooded alluvial bottomland of Rowanta Creek near Rowanta, *Fernald and Long, 8015C,* 8 June 1938, (GH); Sphagnous Creek 4 mi S of Rowanta, *Harvill, 31162,* 1 June 1974, (FARM), Rowanty Creek near SR 703 Bridge, *Wieholdt, 6036,* 5 June 1986, (WM); *Musselman and Musselman, 13007C,* 12 June 1992; South of SR 666 crossing of small, unnamed tributary of Stony Creek SE of 667 and 666 junction, *Musselman and Knepper, 13014C,* 25 June 1992, (ODU); In Stony Creek at SR 666 crossing, *Musselman and Knepper, 13015A* 25 June 1992, (ODU), ESSEX: Tidal brackish water above Chance, *Svenson, 463,* 23 July 1966, (FARM); Tidal shore of Rappahannock River, NE of Loretto, *Fernald and Long, 13855,* 15 October 1941, (GH) FAIRFAX: *Freeman, sn,* 8 August 1925, (FARM); Hunting Creek, SW of Alexandria, *Shull, 200,* 11 August 1902 (NA); Pebbly shore of Occoquan Bay, *Blake, 11521,* 30 July 1932, (GH); Tidal shore of Potomac River, *Blake, 11088,* 20 October 1929, (NA); GLOUCESTER: Piney Swamp, 5 mi NW of Gloucester Point, *Harvill, 12444,* (VPI); GREENSVILLE: Low woods on Fontaine Creek S of Paris Store, *Harvill, 16613,* 16 June 1967, (FARM); Bare mud in cypress swamp along Fountain's Creek at SR 625 bridge, *Musselman and Knepper, 13017A,* 25 June 1992; ISLE OF WIGHT: Villine's Swamp at SR 620, *Musselman and Knepper, sn,* 23 June 1993 (ODU); KING AND QUEEN: Mattaponi River near Walkerton, *Musselman, 13004,* 8 June 1992; (ODU), Mattaponi River about 0.5 mi W of the mouth of mantapike Creek, *Vascott and Ware, 869,* 11 December 1983, (WM); KING WILLIAM: Muddy margin of river, 1 mi SW of Lester Manor, *Mikula, 2355,* 7 July 1976, (FARM); Fresh tidal shore of Mattaponi River, at Horse Landing, near King William Courthouse, *Fernald and Long, 11508,* 14 and 16 October 1939, (GH, US); Mattaponi River, near Scotland Landing, *Wieboldt, 4579,* 12 October 1982, (VPI); Mattaponi River at Wakema, *Wieboldt, 4732,* 26 June 1983, (VPI); MECKLENBURG: Shallow ponds in Forest, W of US 15 near Charlotte Co. line, *Knepper and Musselman, 139401,* 17 January 1994 (ODU) [not mapped]. NANSEMOND: Muddy tidal margin of Blackwater River, Milk Landing, south of South Quay, *Fernald and Long, 11217,* 22 September 1939, (GH); NEW KENT: Fresh tidal shore of Chickahominy River, Lanexa, *Fernald and Long, 13508,* 13 September 1941, (GH, US); Fresh tidal shore of Chickahominy River, Graves Landing, north of Holdcroft, *Fernald and Long, 13507,* 10 September 1941, (GH); Chickahominy River at ST 618 bridge, *Musselman and Musselman, 13025A,* 27 June 1992, (ODU); Bank of Chickahominy River, SE of Providence Forge, *Harvill, 16994,* [sn], (NCU). STAFFORD: Tidal shore (fresh) of Aquia Creek, *Svenson, sn,* 28 August 1963, (FARM); SOUTHAMPTON COUNTY: Sandy tidal margin of Nottoway River, Knight Seine Beach (Battle Beach), *Fernald and Long, 10082,* 23 June 1939, (GH); Wooded bottomland, Three Creek, SW of Grizzard, *Fernald and Long, 10080,* 14 June 1939, (GH, US); North bank of Three Creek near Drewryville at 659 bridge, *Musselman and Knepper, 13009C,* 16 October 1992, (ODU); Sandy alluvial bottomlands of Three Creek, Drewryville, *Fernald, Long, and Smart, 5588A, C,* 22 and 23 June 1936, (US); N of 635 bridge over branch of Seacock Swamp, *Musselman and Musselman 13006C,* 12 June 1992, (ODU); SURRY: Bottomland woods along Blackwater River, about 1 mi SE of Dendron, *Fernald and Long, 12915A,* 14 June 1941, (GH); SUSSEX: Woods by Nottoway River, SE of Lamb's, *Fernald and Long, 12222A,* 18 July 1940, (US); Wooded bottomland, Jones Hole Swamp. W of Coddyshore, *Fernald and Long, 10081,* 16 June 1939, (US).

7. *Isoëtes virginica* N. Pfeiffer. Virginia Quillwort. HABITAT: Granite outcrops HABIT: Amphibious. CORM: Strongly two-lobed; 2 cm in diameter. LEAVES: Light green, stiff, up to 15 cm; not evergreen. Phyllopodia present. SPORANGIA: Round; pigmented. VELUM: Covers 50% or less of the sporangium. MEGASPORE SIZE AND SHAPE (Fig. 8A & B): 438 (396–462) microns. PROXIMAL ORNAMENTATION:

Fig. 8. *Isoëtes virginica.* A) Megaspores. Bar = 500 microns. B) Proximal view of megaspores. Bar = 200 microns. A&B *Musselman and Knepper, 139305A.* C) Microspores. Bar = 100 microns. D) Microspores. Bar = 20 microns. C&D *Musselman and Knepper, sn,* Franklin County, North Carolina.

Tuberculate to short rugulate. DISTAL ORNAMENTATION: Tuberculate with infrequent rugae. MICROSPORE SIZE: 31 (28–34) microns. MICROSPORE ORNAMENTATION: Spinulose (Fig. 8C & D); dark brown in mass. CHROMOSOME NUMBER: 2N = 22, 44 (Matthews and Murdy, 1969).

Isoëtes virginica is the common quillwort of the granite outcrops in the southeastern United States. It is abundant on granite outcrops just across the state line in North Carolina but the Fines Mills Creek collection is the only site for this species in Virginia.

The name *I. virginica* is being used to comply with the treatment of Taylor et al. (1993) in Flora North America. We have studied the type specimen of *I virginica* and though the type is in poor condition, it is indistinguishable from *I. melanopoda.* Therefore, we suggest that *I. virginica* be included in *I. melanopoda.* This will require nomenclatural changes regarding the application of the name *I. piedmontana* (N. E. Pfeiffer) C. F. Reed as this taxon was first described as *I. virginica* N. E. Pfeiffer var. *piedmontana* Pfeiffer.

The plant generally known in the Southeast as *I. piedmontana* is distinguished from *I. melanopoda* by having megaspores which are tuberculate (cf Fig. 5A & B and 7A & B). The megaspores of *I. melanopoda* are the most variable in size (see Table 2) while *I. virginica* has megaspores which are among the most uniform in size. In addition, the phenology and habitat of the two taxa are different. *Isoëtes virginica* matures in the early spring while *I. melanopoda* matures in June and July. *Isoëtes melanopoda* is never found

on granite in Virginia; *I. virginica* is the common quillwort of granite outcrops in the Southeastern United States.

SPECIMENS EXAMINED. — POWHATAN: Granite outcrop. Fines Mills Creek, *Wieboldt, 874,* 28 May 1972, (WM); *Knepper and Musselman, 139305A,* 28 May 1993 (ODU).

ACKNOWLEDGEMENTS

We wish to thank Professor Donald M. Britton for nurturing this work for several years by helping with determinations, spore measurements and general encouragement. It is perhaps ironical that one of the findings of our research is the abundance of *Isoëtes x brittonii!* Carl Taylor reviewed the manuscript and provided the protocol for chromosome analysis. Dan Brunton also read the manuscript and offered suggestions. Jim Allison stimulated us to look more closely at some species. Jim Matthews generously supplied living material for study. Tom Wieboldt provided locality data. We are thankful to Joe Winstead for information on earlier work with *Isoëtes* and a helpful bibliography. Mike Adam of the Electron Microscopy Laboratory at Old Dominion University prepared and photographed spores. Debbie Miller prepared the plates for publication.

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REVIEW

Flora Europaea: Vol. 1: Psilotaceae to Platanaceae by T. G. Tutin, et al. 2nd ed. 1993. ISBN 0-521-41007-X, Cambridge University Press, 40 West 20th St., New York, NY 10011-4211. $200.00

First published in 1964; subsequent changes in taxonomy, nomenclature, and floristics warranted additions and deletions that justified a second edition. Pteridophytes are treated on pp. 1–33, classified into 25 families, 52 genera, and 169 species. Format and style of 1st edition retained with terse keys, brief descriptions, general range statements, and country occurrence lists; illustrations and maps of species are lacking. A useable reference to pteridophytes of Europe. As this work strongly influenced decisions on format and style of the Flora North America Project, it is easy to make comparisons/contrasts between the pteridophyte volumes of these multivolume works. This volume is a bit pricey for pteridologists who might want only the first 33 of 581 pages. — JAMES H. PECK, Department of Biology, University of Arkansas at Little Rock, Little Rock, AR 72204.

Shorter Notes

Flavonoid Glycosides in the Fern *Blechnum orientale* Linn. — The most widespread fern of the moutain region of Gunung Ledang was collected and identified as *Blechnum orientale* Linn. Voucher specimen is deposited at the herbarium of the Department of Biology, Universiti Pertanian Malaysia. Flavonoid content of some *Blechnum* species was reported by Harborne (Phytochemistry 5: 589–600, 1966) who isolated gesnerin and luteolinidin-5-glucoside in *Blechnum brasiliense* var. *corcovadense*. Crowden and Jarman (Phytochemistry 13:1947–1948, 1974) identified 12 deoxyanthocyanins based on apigeninidin and luteolinidin from the leaves of *Blechnum procerum*. (2S)-5,7-dihydroxy-4'-methoxy-6, 8-dimethylflavanone have been identified before in the fern *Matteucia orientalis* by Fujise (Chem. Abstract 24: 3238, 1930). Subsequently, Miraglia, De Padua, Mesquita and Gottlieb (Phytochemistry 24: 1120, 1985) found this (2S)-5,7-dihydroxy-4'-methoxy-6,8-dimethylflavanone in the leaves of *Blechnum regnellianum*. However, no previous reports on the chemical composition of *B. orientale* were found. Thus, leaf flavonoids of *B. orientale* were extracted in order to determine whether or not other species of the Blechnaceae has flavonoid profiles similar to the *Blechnum* ferns previously studied.

Chromatographic profiles from 80% methanolic extracts of leaves were developed via two dimensional paper chromatography. Standard chromatographic procedures were used for examining flavonoids present in direct and acid hydrolysed leaf extracts (Harborne *Comparative biochemistry of the Flavonoids,* 1967; Mabry, Markham and Thomas *The systematic identification of flavonoids,* 1970). Flavonoid aglycones were identified by Rf_s, colour reactions, spectral properties and chromatographic comparison with standard markers (Harborne, *Comparative biochemistry of the Flavonoids,* 1967). Sugars were identified using paper chromatography (Harborne, *Phytochemical methods,* 1984). Known flavonoid glycosides were identified on the basis of Rf_s, UV spectral analysis, acid hydrolysis to aglycone and sugar and by direct comparison with authentic markers. Isolated flavonoids in *B. orientale* revealed the presence of 15 UV absorbing flavonoid spots and the results are presented in Table 1.

B. orientale produces both flavones and flavonols with *O*-glycosidic combination at 4'-and/or 7-, and 3-and/or 7- positions. Kaempferol-3,7-diglucoside have been found before in *Asplenium platyneuron, A. rhizophyllum, A. montanum* (Harborne, Williams and Smith Biochemical Systematic and Ecology 1: 51–54, 1973) and in *A. bulbiferum* (Imperato, Biochemical Systematic and Ecology 17: 161–166, 1989). Luteolin-7-glucuronide was found before in *Pyrrosia serpens* (Markham and Andersen, Phytochemistry 29:3919–3920, 1990); genkwanin and acacetin were reported in *Notholaena nivea* var. *nivea* (Wollenweber, Doerr, Waton and Favre-Bonvin, Phytochemistry 33:611–612, 1993), whereas quercetin-3-glucuronide was found in *Asplenium cuneatum* and *A. capillus-veveris*(Akabori and Hasegawa, Botanical Magazine of Tokyo 82:294–297, 1969). However, other flavonoid glycosides were reported for the first time in the genus *Blechnum*. In addition the presence of *O*-glycosides of flavonols and flavones in *Blechnum orientale* are of chemosystematic interest at the specific level since, these type of flavonoids have not been reported before in the *Blechnum* species. A survey of *Blechnum* species from other parts of the world would obviously prove valuable in the study of the genus to corroborate the results presented here. The author thanks the IRPA 6: 4-07-05-013-J04 for financial support. — Umi Kalsom Yusuf, Department of Biology, Faculty of Science and Environmental Studies, Universiti Pertanian Malaysia, 43400, Serdang, Selangor, Malaysia.

Table 1. Identity and Rf_s values of the Flavonoids in *Blechnum orientale*

Flavonoids	Colours UV/UV+NH_3	BAW	H_2O*	R_fs 15% HOAc	PhOH	80%MeOH	NaOAc	UV spectrum H_3BO_3	NaOH
Quercetin-3,7-digalactoside	Dk/Yg	27	26	40	57	350, 286.5sh., 270	370,273	359.5,270.5	404.5, 327.5sh., 276
Kaempferol-3,7-digalactoside	Dk/Yg	25	88	73	65	352.5, 288sh., 269	379, 273	352, 292sh., 268	396.5, 326, 276
Quercetin-7,4'-digalactoside	Dk/Dk	58	23	33	83	354.5, 269, 256sh.	379,272	372, 261.5	$397_{(dec.)}$,272.5
Luteolin-7-glucuronide	Dk/Yg	55	69*	71	87	352, 297sh., 268	368, 300sh., 267	352,273	396,326.5, 301sh., 273.5
Apigenin-7-glucuronide	Dk/Yg	60	11*	30	86	333.5, 300sh., 270	343.5, 305.5sh., 272	354, 300 sh. 260	390, 274.5
Isorhamnetin-3-glucoside	Dk/Yg	71	39	24	17	353.5, 289sh., 269	370, 305sh., 273.5	352.5, 291, 267.5	405, 321,276.5
Apigenin-7,4'-diglucoside	Dk/Dk	32	50	61	68	331,301sh., 266	341,273,299sh.	347, 297sh., 264	$351.5_{(dec.)}$298sh., 271.5
Quercetin-3, glucuronide	Dk/Y	69	29*	16	39	354.5, 288.5sh 266.5, 259sh	377, 272.5	365, 292sh., 263	385, 273
Quercetin-3, 4'-diglucoside	Dk/Dk	44	39	61	48	357, 269, 257	373.5, 272	370, 262, 291sh.	$379_{(dec.)}$275, 294.5sh
Kaempferol-3, 7-diglucoside	Dk/Yg	26	32	75	43	358, 272.5	377, 273	361.5, 265.5, 288sh	389, 273.5
Quercetin-3',4'di metyl ether-3-glucoside	Dk/Dk	72	68	56	83	349, 269, 291sh.	362.5, 273, 300sh.	348, 295.5, 268	$390_{(dec.)}$,296sh. 274, 323sh.
Acacetin-7-galactoside	Dk/Dk	81	53	36	60	332, 269, 299sh.	341, 272, 302.5sh	349, 265, 298sh	$356.5_{(dec.)}$,273 305.5sh.
Kaempferol-3,7-diglucuronide	Dk/Yg	46	32*	15	10	358, 272.5	377, 273	361.5, 288sh., 265.5	389, 273.5
Genkwanin-4'-glucuronide	Dk/Dk	73	52*	60	65	330, 269	332, 272	343, 269	$345_{(dec.)}$,270

Key: Dk/Yg = dark/yellow green; Dk/Dk = dark/dark; Dk/Y = dark/yellow; sh = shoulder; BAW - butanol-acetic-acid-water (4:1-5); *Glucoronide give streaky spot in H2O and their Rfs are rather variable in this solvent.

The Clute Holotypes and the Pteridophyte Herbarium of Willard Nelson Clute in the Friesner Herbarium of Butler University (BUT). — The herbarium of Willard Nelson Clute is housed in the Friesner Herbarium (BUT). Clute, co-founder of the American Fern Society, was a staff member at Butler University in the 1920's and 30's. His collection of ferns and fern allies from the turn of the century is significant for its selected regional coverage and for type specimens of taxa described by Clute and other fern specialists of the day. Its 1200 sheets consist of 39% Clute specimens along with 131 other collectors with whom he presumably exchanged. Clute provided a brief autobiography (Clute, 1902. Fern Bull. 10:122–123.). He wrote textbooks and popular guides to ferns and flowering plants and tended to wax poetical in his informal writings. Several examples of his work were highlighted in the *Fiddlehead Forum* (Debono, 1989. Fiddlehead Forum 16:24–25). In 1928, at age 59, he took a position as professor of Botany and curator of the Botanical Garden at Butler University in Indianapolis. Clute was a self-described conservative in botanical and nomenclatural matters. While recognizing the necessity for the occasional description of a new species, he steadfastly

Table 1. Fern taxa described by Willard Nelson Clute. Herbaria where types are deposited are indicated if known.

Aspenium clutei Gilbert
Fern Bulletin 8:62–63. TYPE: JAMAICA. Newhaven Gap, altitude 5,600 ft., 9 Feb. 1900, *W.N. Clute 118.* BUT.

Cystopteris fragilis f. *magnasora* Clute
Fern Bulletin 9:65. TYPE: U.S.A. PENNSYLVANIA. Great Bend, 10 Aug. 1900, *W. N. Clute s.n.* NY.

Cystopteris fragilis subsp. *tenuifolia* Clute
Fern Bulletin 16:76. TYPE: U.S.A. ARIZONA. Carr Canyon Huachuca Mts., Nov. 1907, *J. H. Ferris sn.* Lost or location unknown.

Dicksonia pilosiuscula f. *schizophylla* Clute
Fern Bulletin 10:87. TYPE: U.S.A. CONNECTICUT. Andover, 27 July 1901, *A. Vincent Osmun s.n.* Lost or location unknown.

Dryopteris gilberti Clute
Fern Bulletin 8:67. TYPE: JAMAICA. Cuna Cuna Gap, 7 March 1900, *W. N. Clute 200a.* K.

Equisetum ferrissi Clute
Fern Bulletin 12:20. TYPE: U.S.A. ILLINOIS. Joliet. 18 Aug. 1903, *W. N. Clute s.n.* Lost or location unknown.

Lycopodium adpressum f. *polyclavatum* Clute
Fern Bulletin 9:9. TYPE: U.S.A. NEW YORK. Southern end of Staten Island, no date. *W. H. McDonald s.n.*. Lost or location unknown.

Lycopodium alopecuroides var. *polyclavum* Clute
Fern Bulletin 9:9. TYPE: U.S.A. NEW YORK. Southern end of Staten Island, 4 Oct. 1900, *W. N. Clute s.n.* BUT.

Nephrodium cristatum clinotonianum f. *silvaticum* Clute
Fern Bulletin 16:13. TYPE: U.S.A. PENNSYLVANIA. Delaware County, no date, *W. A. Poyser, Type No. 534.* Lost or location unknown.

Polypodium prolongilobum Clute
Fern Bulletin 18:97. TYPE: U.S.A. ARIZONA. Southern slopes of Mt. Lemmon, Santa Catalina Mts., Oct. 1910, *J. H. Ferriss s.n.* US.

Polystichum acrostichoides f. *recurvatum* Clute
Fern Bulletin 12:79. TYPE: U.S.A. CONNECTICUT. Salisbury, no date, *Mrs. C. S. Phelps s.n.* Lost or location unknown.

Pteris aquilina var. *pseudocaudata* Clute
Fern Bulletin 8:39. TYPE: U.S.A. NEW YORK. Babylon, Long Island, 8 Sept. 1898, *W. N. Clute 339.* NY.

opposed the giving of specific rank to "mere forms" (Clute, 1902). Gilbert and others of the day named many forms, most of which are not recognized as significant entities deserving of taxon recognition today (Lellinger, 1985. A field manual of the forms and fern allies of the United States and Canada, Smithsonian Distribution Press, Washington, D.C.). Names of at least two taxa described by Clute are still in use today: a variety of bracken, *Pteridium aquilinum* var. *pseudocaudatum* (Clute) Heller (Fern Bulletin 8:39), and the Jamaican fern *Dryopteris gilberti* Clute (Fern Bulletin 8:67); (Lellinger, 1985; Proctor, 1985. Ferns of Jamaica, a guide to the pteridophytes. British Museum Pub. No. 895). In his *The Fern Allies* (Clute, 1905. Frederick Stokes Co., NY.) Clute proposed several new combinations for species of *Isoëtes* and *Lycopodium* that are identified as synonyms in one recent listing of pteridophyte synonymy (Kartesz & Kartesz, 1980. A synonymized checklist of the vascular flora of the United States, Canada and Greenland, University of North Carolina Press, Chapel Hill, NC). Type specimens of taxa described by Clute are presented with citations in Table 1. A number of names published in the *Fern Journal* but not formally described are listed in Table 2.

Table 2. Names suggested by Clute in the Fern Bulletin, but not formally described (labels may indicate specimen is a type).

Adiantum tricholepis f. *glabrum* (vol. 12:44), US
Asplenium ebeneum f. *furcatum* (vol. 17:88)
Lycopodium lucidulum f. *occidentale* (vol. 11:13), US
Lycopodium volubile var. *angustum* (vol. 8:90), BUT
Osmunda cinnamomea f. *angusta* (vol. 17:12)
Osmunda cinnamomea f. *cornucopiafolia* (vol. 16:109)
Osmunda cinnamomea f. *trifolia* (vol. 17:12)
Osmunda regalis f. *linearis* (vol. 17:10)
Polypodium vulgare var. *perpusillum* (vol. 18:97), US
Polystichum acrostichoides f. *gravesii* (vol. 20:49)
Polystichum acrostichoides f. *lanceolatum* (vol. 20:24)

Unfortunately, most clute holotypes are not present in BUT and were not located through inquiries to other herbaria known to have Clute specimens (E, F, G, ILL, IK, MO, NY, P, RM, and US). It is possible that they were taken by Clute when he left Butler and were subsequently lost (Lellinger, pers. comm.). The Director of BUT requests that workers locating these misplaced Clute holotypes inform the author or Dr. Lellinger.

Butler undergraduate student Jennifer Copeland compiled specimen label information into a database that greatly eased investigations into the collection. Herbarium Assistant Nancy Andrew also assisted. David Lellinger provided a helpful review of the manuscript. — REBECCA W. DOLAN, Department of Biological Sciences, Butler University, Indianapolis, IN 46208.

INFORMATION FOR AUTHORS

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AMERICAN FERN JOURNAL 84(3):73-85(1994)

The Hawaiian Species of *Cibotium*

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Department of Botany, Bishop Museum, Honolulu, HI 96817-0916

The genus *Cibotium* (Dicksoniaceae) comprises approximately ten species ranging from Assam to China and Papua New Guinea in the Old World, to Central America and Mexico in the New World, and Hawaii. The genus is distinguished from *Dicksonia* by the cartilaginous texture of the valves of the indusium, which are strongly differentiated from the lamina. This paper will review the Hawaiian species of *Cibotium.* Hybrids between the Hawaiian species are known and will be discussed in a subsequent paper.

The Hawaiian flora is noted for its many polymorphic species, and the *Cibotium* species are excellent examples. There are few clear-cut characters that separate the species, and while two of them *(C. menziesii* and *C. glaucum)* do have characteristics that are prominent and helpful, the other two taxa have more subtle and variable characters, especially the nature and color of the hairs on the abaxial surface of the segments.

In attempting to understand *Cibotium* in Hawaii, it became obvious that serious problems existed in the clear definition of the species, how many there are, their nomenclature, and their distribution. The original descriptions are incomplete, sometimes do not correlate with the type specimens, and allow for overlapping interpretations. The resulting nomenclatural confusion has caused authors to assign the wrong name, or to identify incorrectly the plant being discussed (Wick & Hashimoto, 1971; Ripperton, 1924). Because of this confusion, the Hawaiian species of *Cibotium* have been seriously misunderstood (Rock, 1913; Skottsberg, 1942; Ogura, 1930).

Another troublesome problem has been that herbarium specimens of *Cibotium* are frequently inadequate for identification, often consisting of no more than a single pinna. At the minimum, a good collection must include the base of the stipe, with its pubescence, as much of the stipe as possible, a middle pinna, and also the distal portion of the frond. Of value also is a basal pinna, including the adjacent stipe, and a crozier, if available. The stipe and crozier can easily be cut in half longitudinally to assure easier pressing, faster drying, and the use of less herbarium space. An adequate single collection may fill as many as three to five herbarium sheets. The practice of collecting only a single pinna for an herbarium specimen should be abandoned, since it will often have little taxonomic value.

TAXONOMIC HISTORY

Cibotium was published by Kaulfuss when he described *C. chamissoi* in April of 1824. (The name *Cibotium* is derived from the Greek word *kibotion,* the diminutive of *kibotos,* a casket, in reference to the form of the indusium.) In December of the same year Gaudichaud described the same taxon as *Pinonia splendens.* Hooker and Arnott (1832) transferred *Dicksonia glauca* described by Smith in 1808 to *Cibotium,* and they mentioned two states of the plant. The first one had longer narrower, glaucous, more deeply cut pinnae. This is the plant today recognized as *C. glaucum.* The description of the other state — not at all glaucous, more coriaceous, less deeply pinnatifid, with broad, rounded sinuses, that do not extend half way to the midrib, and larger in all its parts — fits that of *C. menziesii,* later described by Hooker in 1846.

Descriptions of other Hawaiian cibotiums appeared in the following sequence; *C.*

pruinatum Mett. ex Kuhn (1869), *C. hawaiense* Nakai & Ogura (1930), *C. st.-johnii* Krajina (1938), and *C. nealiae* O. Degener (1952).

Hillebrand (1888) included three species, *C. menziesii* Hook., *C. chamissoi* Kaulf., and *C. glaucum* (Sm.) Hook. and Arn., in his treatment of *Cibotium.* These species were recognized by most authors (Hooker, 1846; Brackenridge, 1854; Smith, 1875; Heller, 1897; Robinson, 1912; Rock, 1913; MacCaughey, 1916; Ripperton, 1924; Christensen, 1925; Degener, 1930) until 1942, when Skottsberg (1942) incorrectly changed the names of *C. chamissoi* (which became *C. splendens*) and *C. menziesii* (which became *C. chamissoi*). Common usage and the literature (Carlquist, 1970; Degener & Hatheway, 1951; Joe, 1964; Porter, 1972; Lamoureux, 1976; Becker, 1976 & 1984) have perpetuated Skottsberg's concepts. However, Joe (1964) states that the horticultural trade generally uses the older, correct names for these species.

By the 1908's local naturalists had accepted six species of Hawaiian *Cibotium,* but their differences were unclear. My own investigations have resulted in acceptance of four species in Hawaii: *C. menziesii, C. chamissoi, C. glaucum,* and *C. nealiae.* Table 1 compares the historical treatments of the *Cibotium* species.

Table 1. Historical comparison of Hawaiian *Cibotium* nomenclature.

Prior to 1942 (Hillebrand)	1942 (Skottsberg)	1951 (Degener)	1994 (Palmer)
C. glaucum	*C. glaucum*		*C. glaucum*[1]
C. menziesii	*C. chamissoi*		*C. menziesii*[2]
C. chamissoi	*C. splendens*		*C. chamissoi*[3]
	C. nealiae	*C. nealiae*	*C. nealiae*

[1]Includes *C. st.-johnii* Krajina (1938)
[2]Includes *C. pruinatum* Mettenius (1869)
[3]Includes *C. hawaiense* Nakai & Ogura (1930)

Characters Used in Classification

The characters used in the classification of Hawaiian *Cibotium* are summarized in Table 2.

Caudices

The height and diameter of the caudex is variable, from 0.5—7 m tall and (10–)16(–90) cm. in diameter. *Cibotium nealiae* is characterized by having a short caudex (0.5 m.), *C. chamissoi* by a narrow caudex, and *C. menziesii* by a tendency to produce a very large, thick caudex when old. While all species can produce adventitious buds along the caudex, this tendency is most prominent in *C. glaucum* in which it is frequent, particularly if the caudex is not vertical.

Stipes

The distribution, color, and type of hairs, or *pulu*, on the stipe are important taxonomic characters. The hairs are of three types. The most common hair is a woolly hair, tan-gray, golden or mustard colored, composed of cells that have collapsed, usually with alternate cells flattened at 90° angles. Straight hairs made up of tubular cells, usually dark brown but sometimes reddish or light tan, are characteristic of *C. menziesii.* The third and most distinctive hair, also associated with *C. menziesii,* is very thick, dark, waxy, and shorter than the straight hairs; it breaks off readily leaving a hard dark tubercle on the stipe.

The pulu may be limited to the base of the stipe or may cover it entirely. Occasionally some hairs are found that differ from the ones typically for the species; these, however, are uncommon and appear to be of no taxonomic significance.

Stipe length and diameter are variable and are probably related more to environment than heredity, since the tree ferns growing in sheltered, moist areas tend to have very large fronds, while those in open, exposed conditions tend to be much smaller and more compact in all respects.

All Hawaiian cibotiums have light colored, linear pneumatophores running along the length of the stipes, and stipes of all species are sulcate adaxially. The pneumatophores are especially prominent when a plant has a dark stipe (e.g., *C. nealiae),* but are obscured when the stipe is hairy. All species can retain old stipes and fronds, but the retention of dried dead fronds as a skirt around the apex of the caudex is particularly characteristic of *C. chamissoi.* Stipe color varies with exposure in some taxa. *Cibotium nealiae* often has very dark stipes when it grows in shade, and the other species may sometimes also have dark stipes, especially in exposed situations. *Cibotium menziesii* and *C. glaucum* generally have stramineous stipes that are sometimes concealed by hairs.

Table 2. Characters useful to distinguish Hawaiian *Cibotium*

Character	*C. chamissoi*	*C. glaucum*	*C. menziesii*	*C. nealiae*
Caudex				
Diameter	Narrow, ca. 12 cm	Medium, ca. 16 cm	Wide 16 cm+	Medium, ca. 16 cm
Height	1–5 m	1–3	1–7 m	0.5 m
Stipe Hair				
Distrib.	At base only	At base only along entire stipe	Along with entire stipe	At base only
Form	Woolly	Woolly	Straight	Woolly
Color	Golden to mustard	Golden, tan, reddish-brown, to gray	Dark brown to reddish-brown, to red to blond	Golden to mustard
Dark, thick waxy hairs	No	No	Yes	No
Hair cells	Flattened	Flattened	Tubular	Flattened
Segments				
Width	4–5 mm	4–5 mm	5–9.5 mm	5–9.5 mm
Abaxial surface	Dull green to slightly glaucous	Glaucous to very glaucous	Light green to slightly glaucous	Moderately glaucous
Abaxial hairs	Arachnoid, tan	Arachnoid, white	Minute punctae, tan	Minute punctae & short arachnoid, tan
Margins	Obtuse, entire	Obtuse, entire	Obtuse, entire	Crenulate, often with cartilagenous rim
Auriculate	No	Yes	No	No
Sinuses				
Depth	Deep, cut 7/8+ to pinna costa	Deep, cut 7/8+ to pinna costa	Shallow, cut 1/3 to 2/3 to pinna costa	Shallow, cut 1/3 to 2/3 to pinna costa
Angle of sinus	Narrow	Narrow	Wide	Wide

Laminae

The length of the lamina, which varies greatly within each species, ranges from less than a meter in more exposed, drier areas, to 3.5 m in moist, protected areas. The number of pairs of pinnae varies greatly with the size of the fronds, which are usually bipinnate-pinnatifid, or tripinnate proximally in larger fronds.

Segments

Features of the segments are especially valuable in separating the species. Segments may be narrow and linear or wide and obtuse, crenulate or entire, and glaucous or dull green abaxially. Prominent yellow veins are visible on the adaxial surface in *C. nealiae,* especially in dried specimens.

A very helpful distinguishing characteristic is the presence or absence of hairs on the abaxial surface of the segments, and the form of the hairs when present. *Cibotium glaucum* and *C. chamissoi* have long-arachnoid hairs, *C. menziesii* has tiny tan punctae consisting of minute stellate hair clusters, and *C. nealiae* has both. Arachnoid hairs are white in *C. glaucum,* but tan in other species.

Pinnules

C. glaucum has subfalcate basal segments that produce a distinct pointed auricle on the basiscopic and sometimes the acroscopic side, a characteristic that nicely separates it from the other species. *Cibotium chamissoi* may occasionally show a tendency for auricle formation, but the auricle is low and rounded and never as prominent or as pointed as in *C. glaucum.*

Sinuses

The shape of the sinuses of the sterile pinnae separate the Hawaiian cibotiums into two groups: one with the sinuses cut nearly to the costa, and the other with sinuses cut only 1/3 to 2/3 the distance to the costa. The deeply cut sinuses have narrow angles, while the shallow ones have wide angles. Fertile pinnules are more deeply cut than the sterile ones, with narrower sinuses and segments. All species have occasional basal pinnae that are tripinnate, especially in very large, fertile fronds.

Spores

Gastony (1982) found that spore size and morphology were sufficiently variable within each Hawaiian species to preclude palynological discrimination between species.

Characteristics found to be of limited value for delineating species include the size and number of sori, the length and width of the frond, and the length and diameter of the stipe. These traits seem to be related to the vigor and maturity of the plant and are probably related to environmental factors.

Key to the Hawaiian Species of *Cibotium*

1. Sterile segments, at middle portion of middle pinna, 5–9.5 mm wide; sinuses cut 1/3 to 2/3 to costa, wide-angled; abaxial surface of segments with minute tan punctae (visible with 20X lens) or with both punctae and arachnoid hairs.
   2. Abaxial surface of segments with minute punctae only; stipe covered with dark brown (sometimes reddish to very light tan) stiff, straight hairs composed of tubular cells; all major islands ..... *C. menziesii*
   2. Abaxial surface of segments with minute punctae and arachnoid hairs; stipe glabrous except very base where a golden to mustard-colored, woolly hair mat is found; Kauai only ..... *C. nealiae*

1. Sterile segments, at middle portion of middle pinna, narrow 4–5 mm wide; sinuses cut 7/8 or nearly to costa, narrow-angled; abaxial surface of segments glabrous or with arachnoid hairs.

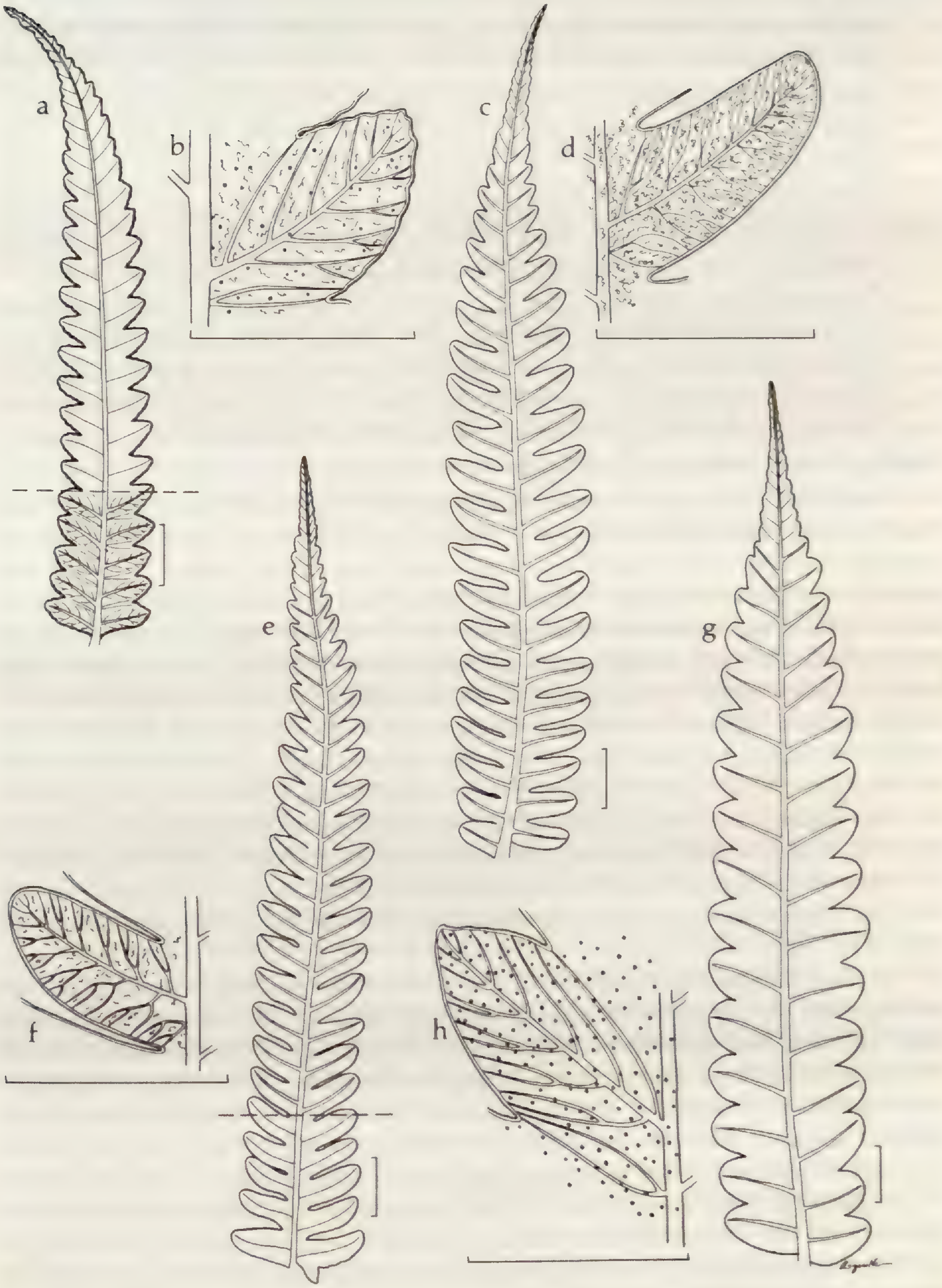

Fig. 1. Pinnules & segments of the four Hawaiian *Cibotium* species. a) *C. nealiae* pinnule, proximal portion illustrating adaxial surface of dried specimen. b) Abaxial surface of segment. c) *C. chamissoi* pinnule. d) Abaxial surface of segment. e) *C. glaucum* pinnule. f) Abaxial surface of segment. g) *C. menziesii* pinnule. h) Abaxial surface of segment.

3. Basiscopic basal segment of pinnule auriculate; abaxial surface of segments glaucous, glabrous to covered with white, arachnoid hairs; stipe glabrous, except at base, to covered with wooly hair; hairs golden to mustard, to reddish brown to dirty gray; hair mat concolorous or streaked .....*C. glaucum*

3. Basiscopic basal segment of pinnule not auriculate; abaxial surface of segments dull green to somewhat glaucous, covered with tan arachnoid hairs; stipe glabrous (young stipes with a fugacious tan scurf) except at base where a golden to mustard-colored mass of woolly hair is found .....*C. chamissoi*

Cibotium chamissoi Kaulf., Enum. Fil. p. 230, fig. 14. Apr 1824. Type: U.S.A., Hawaii, Oahu, S.D., *A. de Chamisso* s.n. (holotype LE, photo BISH!; probably isotype B!) *Pinonia splendens* Gaudich., Ann. Sci. Nat. 3: 507. Dec 1824. – *Cibotium splendens* (Gaudich) Krajina ex Skottsberg, Acta Horti. Gothob. 15:40. 1942. Type: U.S.A., Hawaii, Oahu, 1824 –. *Gaudichaud* s.n. (holotype P, photo BISH!). *Cibotium hawaiense* Nakai & Ogura, Bot. Mag. Tokyo 44:468. 1930. Type: U.S.A., Hawaii, Hawaii Island, Hilo, 29 Mar, 1930, *Ogura,* s.n. (holotype TI!).

Hawaiian name: Hapu´u, on the island of Hawaii-Meu

Caudices 1–2(–6) m tall, diameter variable 10–16 cm.; old fronds retained as a "skirt" near the apex which often has a clothing of golden or mustard-colored hairs. *Fronds* to 5 m, bipinnate pinnatifid to tripinnate at base of large fronds, *Stipes* naked except for the cluster of golden to mustard-colored hairs at extreme base; young stipes covered with a tan, fugacious, scurfy indument of very fine hairs. Linear tan pneumatophores present and inconspicuous. *Rachises* naked except for tan scurf on young fronds. *Laminae* deltate-ovate, coriaceous. *Pinnae* linear-acuminate. *Pinnules* short stipitate, lanceolate. *Segments* narrow and oblong with obtuse apex, basal segments with rounded, non-falcate, non-auriculate base, occasionally having a rounded enlargement of the basiscopic, basal segment suggesting a low auricle, light green to slightly glaucous abaxially, darker green adaxially, abaxial surface of segments with sparse to heavy covering of tan, long, arachnoid hairs that may be absent on older fronds and on older herbarium sheets, brown punctate hair-clusters occasionally seen. Sinuses *acute* and parallel-sided, cut 7/8 or more to costa. *Sori* numerous, usually 8–10+ lining the deeper part of the sinus.

Kaulfuss's original description of *C. chamissoi* and the illustration showing the narrow segments and deep, narrow sinuses are accurate for *C. chamissoi* except for one detail. He stated that both sides of the pinnules were glabrous, and his illustration shows no hairs. However, arachnoid hairs are in fact present on the abaxial surface of the holotype and probably isotype, in spite of the age of the specimens. (I have examined the probable isotype in Berlin and two excellent color photographs of the Leningrad holotype; they are similar and may be two pinnae from the same frond.)

Gaudichaud (1824) published the same species, collected on Oahu, as *Pinonia splendens* and did not mention hairs on the abaxial surface of the segments. However, in his 1826 discussion of this taxon he did mention that the segments are woolly beneath. Photographs of the holotype show that the abaxial surface of the segments is hairy.

In 1942, Skottsberg, on the basis of an incorrectly perceived lack of pubescense on the abaxial surface of the probable isotype at B, applied the name *C. chamissoi* to what is here called *C. menziesii* and created the appellation *C. splendens* for *C. chamissoi*. He

had examined a pinna labeled *C. chamissoi* lent to him by Diels and annotated "Typus! *Cibotium Chamissoi* Kaulf. (Syn. *C. Menziesii)* Hook." by Krajina in 1934. This specimen had been collected and donated by Chamisso, but it was not labeled as a type by Kaulfuss; it is probably an isotype, and has an appearance very similar to the holotype in LE. At the time Skottsberg examined this specimen he believed, correctly, that *C. menziesii* Hooker had an abaxial surface that was glabrous or had only "furfuraceous dots," and, incorrectly, that *C. chamissoi* Kaulfuss was also glabrous, and the *Pinonia splendens* Gaudichaud had arachnoid hairs abaxially. Thus, he applied the name *C. chamissoi,* incorrectly, to what is here called *C. menziesii* and *C. splendens* to what is here called *C. chamissoi.*

The description of *C. hawaiense* (Ogura 1930) (Published as C. hawaiense not C. hawaiiense.) was incidental in an article that was devoted to the anatomy of the caudex and stipe of the genus *Cibotium.* At that time it was generally accepted that *C. glaucum, C. chamissoi* and *C. menziesii* were the only three recognized species of *Cibotium* in Hawaii. Ogura stated that he had collected three species in the Glenwood area on the island of Hawaii. He apparently believed that he had collected *C. chamissoi* and *C. menziesii,* both of which he considered to be very common, and he wrote that *C. glaucum* occurred rarely and was not collected by him. He believed he had collected two known species, that he had not taken *C. glaucum,* and that therefore the third species that he found must be a new species.

Cibotium glaucum, which he thought was rare, is in fact the most common *Cibotium* on the Island of Hawaii (Becker, 1976). This is especially true in the Glenwood area, making up most of the tree fern forest there. I examined the specimens in the Tokyo University Herbarium at Koishikawa (TI), collected by Ogura at Glenwood in 1930, and discovered that he did indeed collect *C. glaucum,* but he had identified it as *C. chamissoi.* He had also collected *C. menziesii,* which he labeled correctly. Since Ogura believed that he had found *C. menziesii* (correctly so) and believed that he had taken *C. chamissoi* (when in reality he had collected *C. glaucum* and wrongly labeled it *C. chamissoi),* and believed that he had not been able to find *C. glaucum* at all, his third collection was construed as a "new species", which he described as *C. hawaiense.* This was, however, the real *C. chamissoi* that he had failed to recognize.

Ogura's description of *C. hawaiense* is entirely consistent with a slightly glaucous, tall form of *C. chamissoi.* The holotype of *C. hawaiense* is unquestionably *C. chamissoi.* Krajina in 1966 had annotated the same sheet as "Typus! subspecies", "*Cibotium splendens* (Gaud.) Krajina subsp. *hawaiiense* (sic) (Nakai & Ogura) Krajina comb. nov.". Krajina thus agreed with my interpretation of this specimen as *C. chamissoi* (as shown above the correct name for *C. splendens* is *C. chamissoi),* although there are insufficient differences to warrant making it a subspecies. *Cibotium hawaiense* Nakai & Ogura is treated here as *C. chamissoi* Kaulfuss and varies only in a tendency to have taller, more slender caudices and a mild glaucouness on the abaxial surface of the segments. Plants of this type are frequent on the north slope of Haleakala and scattered on all the major islands except Kauai. A full spectrum of intermediate forms is seen throughout its distribution, and it is not appropriate to recognize the Hawaii plant as a separate species.

Among the Hawaiian pteridophytes, several endemic species are limited to one or two islands, and *C. hawaiense* has been considered one of these (W.H. Wagner, pers. comm.). With the recognition of this taxon as *C. chamissoi,* there are no endemic ferns

now recognized as restricted to the Island of Hawaii.

Cibotium chamissoi is the most common low elevation tree fern on Oahu. It is often seen up to 600 m, and less often at higher elevations where it is often associated with *C. menziesii* and *C. glaucum*. It is found on all the islands except Kauai, where it has not been collected. In areas where its lowland habitat still exists, *C. chamissoi* can be found as low as 50 meters, usually in moist, shady, protected locations.

C. chamissoi can be recognized in the field by the usual presence of a skirt of dead fronds below the crown of the often slender caudex; older stipes that are naked except at the base; the pale green color of the frond; non-falcate, non-auriculate basal segments of the pinnules; and tan arachnoid hairs covering the abaxial surface of the segments. Young stipes have a fugacious scurf of fine hairs that covers their distal parts.

Specimens Examined: Hawaii: Wright Road, Palmer 554 (BISH). Maui: Kipahulu Valley, West part of valley, 3200 feet, *Lamoureux, Dewreede 4039* (BISH). Oahu: Waahila Ridge, Koolau Mountains, *Palmer 884* (BISH), Moanalua Valley, *Palmer 811* (BISH), Makaha-Waianae Ridge, *Wilson 187* (BISH), Palehua-Waianae Mtns., *H. St. John 11157.*

Cibotium glaucum (sm.) Hook. & Arn. Bot. Beechey Voy. 108. 1832. – *Dicksonia glauca* Sm. in Rees´ Cycl. 11(2): 1808. – Lectotype (chosen here) U.S.A., Hawaii, sin. loc., s. d., Menzies, s.n., (K!)

Cibotium st.-johnii Kratjina Stud. Bot. Čech. 1:94. 1938. – Type: U.S.A., Hawaii, Kauai, Waimea Dist., Na Pali-Kona Forest Reserve, inter vallem fluvii Halekua Stream et locum Kaholuamanu dictum, *Krajina 26* (presumably PRC) *Cibotium st.-johnii* Kratjina subvar. *auritum* Krajina, Stud. Bot Čech. 1:95. 1938 – Type: U.S.A., Hawaii, Kauai, on Kaholuamanu, above Waimea, *Heller 2818* (holotype PRC; isotypes BISH!, UC)

Cibotium st.-johnii Krajina var. *fallax* Krajina, Stud. Bot Čech. 1:96. 1938. – Type U.S.A., Hawaii, Kauai, Waimea Dist., Na Pali-Kona Forest Reserve, Kaholuamanu, vallis fluvii Waialae River, *Krajina 27* (presumably PRC) *Cibotium st.-johnii* Krajina subvar. *crenatum* Krajina, Stud. Bot. Čech. 1:96. 1938. – Type: U.S.A., Hawaii, Kauai, Waimea Dist., Na Pali-Kona Forest Reserve, Kaholuamanu, vallis fluvii Waialae River, *Krajina 27* (presumably PRC)

Cibotium st.-johnii Krajina subvar. *integrilobatum* Krajina, Stud. Bot. Čech. 1:96. 1938 – Type: U.S.A., Hawaii, Kauai, Weimea (=Waimea), Kaunupalanui, *Hochreutiner 3547* (G).

Cibotium chamissoi Kaulf. *ß var.* Hillebrand, Fl. Hawaiian Isl. II(2):547. 1888.

Hawaiian name: Hapu´u pulu.

Caudices 1–2 (–6) m often taller, erect, commonly with buds, especially if the caudex is not upright, diameter 16–18+ cm. *Fronds* up to 7 m. *Stipes* with tuft of matted woolly hair limited to base, to very hairy with entire stipe covered with extremely variable (usually woolly, sometimes straight on very hairy forms) matted hairs, and all intermediate grades of hairiness; color varying from golden, to tan, to mustard, to dusty gray, and occasionally to reddish brown in more hairy forms, color monochrome on some plants and polychrome with streaking on others. Cells of the hairs flattened alternately at 90° angles. The hair cells on very hairy forms are sometimes tubular. Young stipes on less hairy forms covered with a light brown, very fine scurfy mat of fine hairs that is soon fugacious. Stipes 1/3 to 1/2 of length of frond, stramineous, occasionally almost black,

sulcate. Linear white to light tan pneumatophores extend the entire length of the stipe bilaterally. *Rachises* glabrous to covered with woolly hair. *Laminae* up to 4.5 m, usually smaller. Ovate-lanceolate, bipinnate and deeply pinnatifid to tripinnate, texture subcoriaceous. *Pinnae* Linear-acuminate, proximal portions of larger pinnae often tripinnate. *Pinnules* lanceolate-accuminate. *Segments* narrow 4.2–5 mm, linear-oblong, with basiscopic and sometimes acroscopic segments of each pinnule subfalcate and having a projecting, usually pointed auricle that often overlaps the pinna rachis. Abaxial surface very glaucous to dull glaucous (dull green on immature fronds), glabrous to copiously covered with white cobwebby hairs. Veins prominent and forked, arising from central vein of segment, occasionally arising basiscopically from the pinna costa. *Sinuses* narrow angled, cut 7/8 to completely to the costa. *Sori* numerous, usually 4 to 8 (to 14) lining the proximal part of the sinus. *Notes on the species:* In his original description of *Dicksonia glauca* Smith did not designate a holotype. He mentioned that he possessed "only a portion of the middle part of its frond, brought by Mr. Menzies from the Sandwich Islands." A specimen of this description in Kew, mounted in the middle of a sheet between two other specimens of *C. glaucum* and with a note, apparently in Menzies' handwriting, is here designated as lectotype. Smith's description entirely fits this specimen, and the specimen is typical of what is now known as *C. glaucum.*

Cibotium glaucum is extremely variable in its pubescence, and hair color. On the Island of Hawaii, in moist and shady areas, the frond is almost glabrous. As one proceeds to the islands of the northwest the fronds become progressively more hairy, becoming most hairy on the summit ridge of the Koolau Mountains, on Mt. Kaala in the Waianae Mountains of Oahu, and on Kauai in the Kokee and Alakai Swamp areas. The less hirsute forms have only a tuft of hair at the base of the stipe with a fugacious scurf of very fine hairs that extends up the younger stipes. The most hirsute forms on Kauai have thick tangled mats of hair that cover the entire stipe and most of the rachis and also bear tufts of woolly hair on the pinna rachis. There is a complete continuum between the glabrous and hirsute forms, and intermediate plants of all degrees of hairiness can be found. All conditions from glabrous to hirsute can most easily be seen on the Alakai Swamp Trail in Kauai's Kokee State Park.

The hair color varies from gray to tan to brown to reddish, and while it is uniform in most plants, the colors often occur in streaks in the more hirsute forms. Plants with a hairier stipe and rachis usually have more hair on the abaxial surface of the segments, although it is not uncommon for plants with very hairy stipes and rachises to have glabrous abaxial segment surfaces, except over the veins. Very hairy, hairy, and glabrous plants are found on all the major islands.

The size of the auricle is inversely related to the hairiness in that the largest auricles are found on the pinnae of the glabrous plants on the island of Hawaii, auricles are smaller and plants more hairy on the islands toward the northwest.

Cibotium glaucum is a plant of higher elevations. Although it may grow at 50 to 100 meters it is more likely to be seen in greater numbers above 700 meters, and it becomes by far the most common species at higher elevations. Hillebrand (1888), MacCaughey (1916), and Rock (1913) reported it as uncommon, but this is true only at lower elevations.

Cibotium st.-johnii was described by Krajina (1938) as differing from typical *C. glaucum* in the extreme hairiness of its stipe, rachis and costae. He also described two

varieties and three subvarieties of *C. st.-johnsii,* and apparently thought it reasonable to describe plants selected from the continuum of variability in hairiness seen in this taxon at these taxonomic levels. The hairiness, however, represents only an extreme in this single character that would allow for the separation of taxa. Plants of the glabrous form with minimal hairiness grow side by side with very hair forms, and intermediate forms are found in the same area; thus it seems very likely that the various forms interbreed.

An isotype of *Cibotium st.-johnii* var. *st.-johnii* subvar. *auritum* at BISH, is typical of the hairy end of the spectrum of *C. glaucum.* In 1966 Krajina had annotated this same specimen "*C. glaucum* (Sm.) H. & A. subsp. *st.-johnii* (Krajina) c.n. var. *fallax* Krajina." By this time he had evidently reinterpreted the species status of *C. st.-johnii.*

Specimens Examined: Kauai: Kilohana, *Palmer 905* (BISH), Alakai Swamp Trail, *Palmer 906* (BISH). Oahu: Poamoho Trail, Koolau Range, *Krajina 611015253* (BISH), Kaala Summit Bog, *Palmer 734* (BISH). Hawaii: Hawaii Volcanoes National Park, Napau Crater, *Newell 859* (BISH): N.W. end of Kahaluu Forest Res. N. Kona Dist, *St. John 18499* (BISH).

Cibotium menziesii Hook., Spec. Fil. 1: 84, t.29. 1846. – *Dicksonia menziesii* (Hook.) Hook. & Baker, Sn. Fil. 49. 1866. – Type: U.S.A., Hawaii, Oahu, sin. loc., s.d., Menzies Lay & Collie s.n., (Lectotype here chosen K!). *C. pruinatum* Mett. ex Kuhn, Linnaea 36: 150. 1869, *Dicksonia menziesii* Hook. var. *pruinata* Baker (Mett. ex Kuhn), Syn. Fil. 460 Fig 58, 1874.

Hawaiian name: Hapu´u ´i´i

Caudices 2 to 7 m tall, 16 cm – 0.8 m in diameter, budding occasionally. Apex covered with the same type of hairs seen on the stipe. *Fronds* deltate-ovoid, coriaceous. *Stipes* 1/3 to 1/2 of the length of the frond, sulcate. Thickly covered at base with long, dark brown to maroon occasionally reddish straight hairs, composed of tubular cells, and sparsely covered with thicker, shorter, darker, more wiry hairs in more distal portions, except on older fronds where much of the hair was abscised, and on many specimens on the island of Hawaii "blond" (pale tan to whitish) hairs are found on the basal portion of the stipe. Stipes often tuberculate, the tubercles being the residual conical base of shorter, much thicker, dark waxy hairs that have abscised, and are found loose in the finer hairs. Pneumatophores, bilateral, linear, tan and inconspicuous. *Rachises* with same type of hairs as stipe, but these shorter and more scattered, sometimes glabrous. *Laminae* ovate-lanceolate, up to 4 m, usually less, bipinnate-pinnatifid to tripinnate at base of larger pinnae. *Pinnae* linear-oblong. *Pinnules* lanceolate. *Segments* broad 0.5–0.95 cm with rounded base, never falcate or auriculate. Texture leathery, adaxial surface usually dark green, shiny, abaxial surface of segments dull green to mildly glaucous, with many scattered, very small (0.1–0.2 mm.), tan punctae (stellate hair clusters seen with a 20X lens), veins prominent and once to twice forked, arising from the segment vein and basiscopically from the costa, occasionally anastomosing with those of adjacent segments at the base of the segments in larger fronds. *Sinuses* broad and wide-angled, cut 1/3 to 2/3 to the costa. *Sori* 4 to 8^{+} lining basal portion of sinus.

Hooker's description of the fronds as being everywhere glabrous is contradicted by his illustration that shows the abundant, minute punctate hairs on the abaxial segment surface. These dots, that require a 20X lens to see well, are among the most important characters for identifying this taxon. While they are often deciduous on windblown fronds and on older herbarium specimens, some usually remain. Arachnoid hairs are occasional-

ly present but are infrequent, sparse and short. All plants of this taxon have the tan punctae; however, these punctae may sometimes also be found as minor components on the abaxial surface in *C. chamissoi* and *C. glaucum* (in the latter they are white), but in these species the arachnoid hairs are by far the most prominent feature. *Cibotium nealiae* has an approximately equal mixture of tan arachnoid hairs and punctae that are prominent against a moderately glaucous background.

A specimen apparently collected on Oahu by Lay and Collie during Beechey's voyage has been designated as lectotype. It is typical of what is now recognized as *C. menziesii,* and it fits Hooker's description (Hooker, 1846) as well as his illustration of this species.

Cibotium menziesii, with dark straight hairs that cover the entire length of the stipe, a darker, more leathery appearing blade, and with its wider segments with more open sinuses, is readily identifiable in the field. Frequently plants on Kauai and sometimes elsewhere have finer light reddish hair, and plants on Hawaii island have white to tan hairs, but the hairs are always straight. Older weathered fronds lose the hair on the upper portion of the stipes.

The unfurled crozier, with its light colored, naked center of embryonic pinnae, and surrounding coil of dark stiff hairs, is distinct from the other *Cibotium* species.

Most, but not all, plants of this taxon have a type of hair on the stipe that is seen in no other *Cibotium* species in Hawaii (or, apparently, anywhere else in the world.) These hairs are dark, thick and waxy, usually not as long as the other hairs, and they are easily fractured. When broken at their bases, they leave a dark truncated cone, often with a light center; this gives the stipe a tuberculate appearance. In most herbarium specimens these hairs have already broken from their bases and are found loose in the mat of straight hairs.

In the early part of this century individuals of this taxon were known to have a diameter of three feet and a height of 24 feet, not including the fronds, which could be 12 feet long (Rock, 1913). It is rare to find a plant approaching this size today.

The status of *C. pruinatum* has been confusing. Some have called it a distinct species (Heller, 1897; Moore, 1879), and it was treated as a variety of *C. menziesii* (Baker, 1863) or combined with *C. menziesii* (Christensen, 1906; Hillebrand, 1888). In addition Krajina had labeled several herbarium sheets of specimens recognized by me as *C. nealiae* as *C. splendens var. pruinatum.*

Mettenius's description of *C. pruinatum* (Kuhn, 1869) is consistent with a mildly glaucous form of *C. menziesii,* and he stated that the specimen he examined was labeled as *C. menziesii* by Brackenridge. I have been unable to locate the type specimen. *Cibotium pruinatum* Mettenius ex Kuhn is here combined with *C. menziesii* Hooker. It differs largely in having a somewhat glaucous abaxial surface, more acuminate segments and, often, very pale hair on the stipe. However since a full range of intermediate states is now known it would not be appropriate to recognize *C. pruinatum* as a separate species. This form is most common Hawaii island. The punctate hairs on the abaxial surface of the segments stand out against the somewhat glaucous background, and in addition to the punctae a greater number of short arachnoid hairs are found on this form.

On the Island of Hawaii, in the Kilauea area and in the Kohala Mountains, the lower portion of the stipe of this variant is often covered with "blond", pale tan, or mustard col-

ored, straight hairs, while the more typical darker hairs appear farther up on the stipe.

Specimens Examined. Kauai: Alakai Swamp Trail, *Palmer 758* (BISH), Namolokama Plateau, Hanalei District, *Hobdy 3367* (BISH). Oahu: Poamoho Trail, Koolau Mtns., *Palmer 783* (BISH). Maui: Wailau Valley, *Degener 25287* (BISH), Kipahulu Valley, *Lamoureux & DeWreede 4038* (BISH). Hawaii: Kau Forest Res., Wood Valley, *Russ 12* (BISH).

Cibotium nealiae Degener. Fl. Hawaiiensis Book 1: Fam. 12. 1951 Type: U.S.A., Hawaii, Kauai, East of Kapalikea, Hanalei, Feb. 4, 1950, *Degener & Hatheway 20,475,* (Holotype, NY!' Isotype, BISH!)

Cibotium glaucum var. *nealiae* Hatheway. Degener. Fl. Hawaiiensis Book 1: Fam. 12. 1951 Type: U.S.A., Hawaii, Kauai, East of Kapalikea, Hanalei, Feb. 4, 1950, *Degener & Hatheway 20,475,* (Holotype, NY!; Isotype, BISH!)

Caudices short, 0.5 m rarely to 1 m, apex covered with golden to mustard-colored hair. *Fronds* to 6 m. *Stipes* 1/2 the length of the frond dark purple to black in deep shady, moist valleys, stramineous in more open dry areas. Naked except for tuft of golden, occasionally mustard colored, woolly hair only at extreme base, cells of hairs flattened. Linear white pneumatophores on either side of stipe stand out in contrast to the dark stipe and on rachis to the second pinna (not readily visible on dried material). *Rachises* naked. *Laminae* coriaceous, deltate-ovate, bipinnate-pinnaifid. *pinnae* linear-lanceolate, dark green above, pruinose beneath. *Pinnules* linear-acuminate. *Segments* 5–9 mm broad, segments with rounded base, never falcate or auriculate, obtuse, with crenulate margins, commonly with a cartilaginous rim, adaxial surface glabrous, abaxial surface with both tan arachnoid hairs and furfuraceous dots (seen with a 20X lens) that stand out against a moderately glaucous background, veins prominent and yellow on adaxial surface in dried specimens. *Sinuses* wide angled, cut 1/3 to 2/3 to costa, more deeply when fertile. *Sori* 4–8+ lining deeper portion of sinuses.

Cibotium nealiae was described by Degener (1951), but in the same publication Hatheway named the same taxon *C. glaucum var. nealiae. Cibotium nealiae* is here recognized as a wholly distinct species. It appears to be limited to Kauai where it occupies the same ecological niche that *C. chamissoi* does on the other islands. As far as is known the ranges of these two species do not overlap. *C. nealiae* often grows with *C. glaucum* and *C. menziesii,* but frequently occurs as the sole species in large tree fern stands.

This species may be easily recognized in the field by its short caudex, bearing large fronds, that, in shady areas, have a dark purple stipe. Sterile segments of the pinnae are broad, and the sinuses are wide-angled and V-shaped, cut only 1/3 to 2/3 to the costa.

The slightly glaucous abaxial surface bearing both tan punctae and short tan arachnoid hairs aids identification. The venation on the adaxial surface of the segments is conspicuous on herbarium sheets, but is often inconspicuous on the living plant.

Specimens Examined: Kauai: Lihue-Koloa Forest Reserve, *Flynn 1988.263* (BISH), Wainiha Valley, *Wilson 225*(BISH), Limahuli Valley, *Palmer 914* (BISH), Honopu Trail, Kokee State Park, *Palmer 763* (BISH).

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Collection of *Azolla microphylla* in Paraguay and its Characterization by Allozymes and DNA-RAPDs

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Azolla Lam. is a genus of freshwater aquatic ferns which lives symbiotically with a nitrogen-fixing cyanobacterium. There are seven designated species (Lumpkin & Plucknett, 1982), three of which grow natively in the neotropics and subtropics. *A. microphylla* Kaulf. (MI in this paper) is the only one whose range reportedly lies entirely within the tropics. From an arbitrary focal point at the Galapagos Islands, its distribution extends north into the West Indies and Central America, and also across northern and central South America (Schofield & Colinvaux, 1969; Morton & Wiggins, 1971). Within South America, MI is purportedly "centered in the lowlands of Brazil" (Svenson, 1944).

The MI species, or the MI X *A. filiculoides* (FI) hybrid (Van Cat et al., 1989), is also a superior *Azolla* for application as a biofertilizer with irrigated rice. Its rapid growth in hot, humid regions of South and Southeast Asia, combined with greater fungal resistance, makes MI more desirable than the indigenous *A. pinnata* (Maybbayad, 1987; Ventura et al., 1987; Choonluchanon et al., 1988; Liu & Zheng, 1989).

The MI which have become field accessions are stored in the *Azolla* germplasm collection at the International Rice Research Institute (IRRI) in the Philippines. All of the MI accessions from which hybrids and F_1 progeny have been made were collected in northwest Paraguay in 1979. MI 4017 and 4018, among the most heat-tolerant of the tested germplasm, are commonly grown in IRRI fields at Los Baños and South Cobato (Ventura et al., 1987).

A second survey was sponsored by IRRI in 1988 to gather additional agroecotypes of *Azolla*. This paper discusses the genetic results found from that survey.

METHODS

Survey and Collection

Field visits were made in 1988 during the Paraguayan winter (July). The most intensive search for *Azolla* was conducted in the region north of Asuncion where the earlier accessions were found. Germplasm was collected as representative specimens of each area.

Growth and Maintenance

Azolla clones from IRRI were maintained in the germplasm collection at the University of Michigan-Dearborn. Plants were grown with N-free BG-11 nutrient medium (Rippka et al., 1979) under combined natural sunlight and fluorescent lamps (12:12 photoperiod) with a temperature of about 25°C. Spores of one breeding population were germinated in distilled water under natural sunlight with daily agitation, then seedlings were transferred individually into culture with N-free medium.

Allozymes

Enzymes and DNA profiles were completed to ascertain molecular distinctions among *Azolla* of differing phenotypes. Zymograms of newly collected accessions were first obtained in 1989, but repeated during 1991 in an expanded series of trials which included MI from other countries (Table 1). Additionally, surviving *Azolla* accessions from those collected in 1979 in the Gran Chaco were re-evaluated. Controls were included from *A. filiculoides* (FI), *A. mexicana* (ME), and *A. caroliniana* (CA).

Table 1. *Azolla microphylla* from other sources or geographic regions which were also fingerprinted with the new accessions from Paraguay.

IRRI Code	Collector	Site information
4024	Gunapala	Ecuador (Galapagos Islands)
4028	Do Van Cat	Hybrid (4018 X 1001)
4031	Lin	Megasporocarp - MI 347 (FAAS, China)[c]
4052	Ramirez	"Rong Ping" FAAS hybrid (MI X FI)
4139	Ferrera-Cerrato	Mexico (Tabasco)
4501[a]	Myasawa	Brazil (Paraiso do Norte)
4502[a]	Myasawa	Brazil (Querencia do Norte)
4504[a]	Lumpkin	Ecuador (Galapagos Islands)[c]
4506[a]	Ferrera-Ceratto	Mexico (Sinaloa)
4510[a]	Van Hove	Paraguay[c]
MI-2	Uncertain[b]	Ecuador (Galapagos Islands)[c]
MI-3	Uncertain[b]	Paraguay[c]

[a]Duplicates from the *Azolla* collection of the Universite Catholique de Louvaine (Belgium).
[b]Obtained from R. Caudales (Rutgers Univ.) and labeled as accessions from G. Peters (VA Commonwealth Univ.).
[c]4504 originally designated as 4023, 4510 as 4061 (a field sample of 4018), MI-2 as 4504, MI-3 as 4013; the origin of 4031 (MI 347 in the China collection) is unclear.

The techniques for polyacrylamide gel electrophoresis (PAGE) and staining of enzymes which are polymorphic in *Azolla* have been described (Zimmerman et al., 1989a,b). Three acrylamide concentrations – 7.5%T, 9%T, and 12%T (2.5% C) – were utilized with PAGE gels. Certain enzymes required starch gels for greater staining intensity. Those gels contained 11% (w/v) hydrolyzed potato starch (Sigma Chemical Co., St. Louis, MO., USA). Procedures and gel/electrode buffer systems for starch gel electrophoresis were those recommended by the Grain Legume Genetics and Physiology Research Unit, USDA-ARS, Pullman, WA (Muehlbauer et al., 1989).

Thirteen enzymes were stained: alpha-amylase (AMY; EC 3.2.1.1), aspartate aminotransferase (AAT; EC 2.6.1.1), diaphorase (DIAP; EC 1.8.1.4), glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49), isocitrate dehydrogenase (IDH; EC 1.1.1.42), malic enzyme (ME; EC 1.1.1.40), malate dehydrogenase (MDH; EC 1.1.1.37), phosphoglucoisomerase (PGI; EC 5.3.1.9), phosphoglucomutase (PGM; EC 2.7.5.1), 6-phosphogluconate dehydrogenase (6PGD; EC 1.1.1.44), shikimate dehydrogenase (SKDH; EC 1.1.1.25), triose-phosphate isomerase (TPI; EC 5.3.1.1), and xanthine dehydrogenase (XDH; EC 1.1.1.204).

DNA polymorphisms

The procedures for harvesting and purifying DNA from *Azolla* have been described (Zimmerman et al., 1991). Rapid amplified polymorphic DNAs (RAPDs) were obtained by modifying a known protocol (Williams et al., 1990). An estimated 25 ng of genomic DNA was used as template in 25 µl reactions containing 50 mM KC1, 10 mM Tris HC1

pH 9.0, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.1% Triton X-100, 100 µM each dNTP, 1 µM primer, and 1 unit *Taq* DNA polymerase (Promega Corp., Madison WI, USA). The 24 primers (Genosys Biotech., Inc., The Woodlands TX, USA) in this study consisted of 10 base pairs with 50–80% G + C content.

Amplification by the polymerase chain reaction (PCR) was performed on a Perkin Elmer 9600 thermal cycler. Each of the 40 cycles consisted of 20 sec at 94 °C, 1 min. at 36 °C, and 1 min. at 72 °C. Temperature transitions were at maximum speed except for a 3 min. ramp time from 36 to 72 °C. The final step was a 7 min. elongation at 72 °C. PCR product was fractionated on a 2% agarose gel (1% NuSieve GTG plus 1% Seakem LE; FMC Bioproducts, Rockland ME, USA) and stained with ethidium bromide.

Results

Survey and collection

Azolla samples were located in seasonal and artificial ponds, ditches, and an overflow depression near the Rio Paraguay. Most of the fern populations were located north of Asuncion along the Ruta Trans Chaco (Table 2). *Azolla* was found in each of the three soil regions of Paraguay (Fig. 1). Available data from monitoring stations (Ministry of National Defense) showed that all sites bearing *Azolla* have isotherms of 23–25 °C and precipitation ranging from 900–1500 mm annually.

Table 2. *Azolla microphylla* collected in Paraguay during 1979 (by C. Shock) and 1988 (by Watanabe and Zimmerman). Accessions are identified by code number, frond color at time of collection, and locale.

IRRI Code	Frond color	Site information
4001–402	—	Ruta Trans Chaco, Km 167–670 (1979)
4062	red	2 km E of Ypacarai (Co)[a]
4063	red	Ruta Trans Chaco, Rio Paraguay (PH)[a]
4064	red	Ruta Trans Chaco, Km 86 (PH)
4065	red	Ruta Trans Chaco, Km 175 (PH)
4066	green	Ruta Trans Chaco, Km 380 (B)[a]
4067	green	Ruta Trans Chaco, Km 393 (B)[b]
4068	green	1 km W of Loma Plata (B)
4069	green	Campo Esperanza, 22 km E of Loma Plata
4070	green	Laguna Isla Poi, SE of Filadelfia (B)
4071	green	Ruta Trans Chaco, Cruce Toledo (B)
4072	(red)[c]	Ruta Trans Chaco, Fortin Toledo (B)
4073	red	Ruta Trans Chaco, Km 192 (PH)
4074	red	N of Cnl. Oveido, Km 152–154 (Ca)[a]
4075	red	Lago Canada, S of Nueva Italia (Ce)[a]

[a]Key to departments: Co = Cordillera, PH = Pres. Hayes, B = Boqueron, Ca = Caaguazu, Ce = Central

[b]Biomass collected as dried sporocarps

[c]Originally designated a green isolate in the field, but a red isolate (site unknown) was deposited in the IRRI collection.

Two natural phenotypes were discovered (Table 2). The *Azolla* of the Gran Chaco (MI 4066–4072) exhibited mature green fronds. These appeared north of Asuncion and grew luxuriously in monoculture in all locales of its occurrence, often as thick mats. No effects of insect herbivory were found. Sporophytes were similar in color and growth habit to MI 4018, the reference specimen for this species in the IRRI collection. Populations of the other phenotype were found growing less vigorously. They possessed

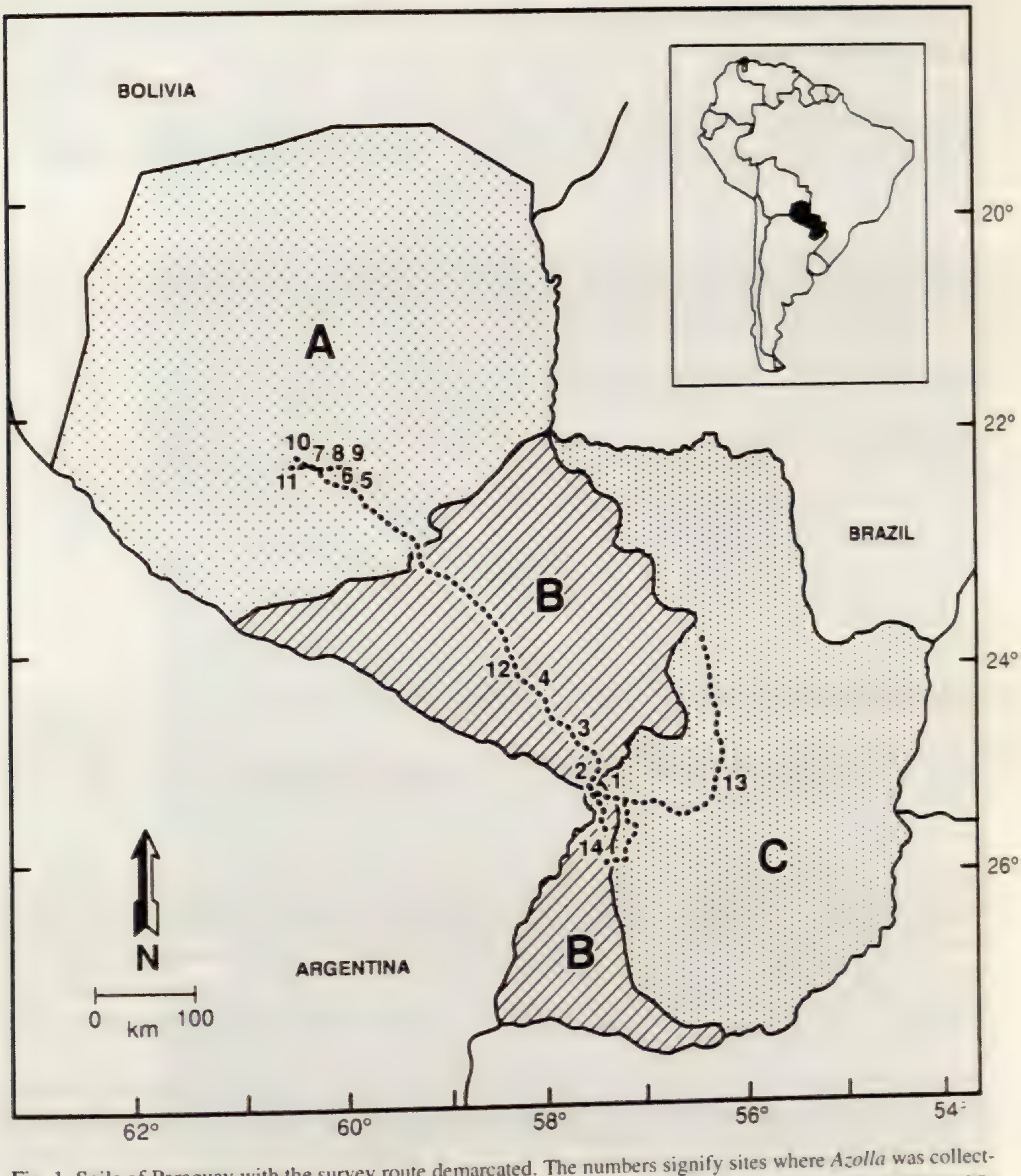

Fig. 1. Soils of Paraguay with the survey route demarcated. The numbers signify sites where *Azolla* was collected (see Table 2). Soils are classified by the system of the Food and Agriculture Organization (FAO/UNESCO, 1968, 1971). Area A = formed by kastanosems together with solonetz. Area B = formed by planosols, gleysols, and fluvisols. Area C - formed by acrisols, luvisols, or nitosols in the east, bordering the Rio Parana..

fragile fronds, red in color on the periphery and reddish-brown in the central portion. These *Azolla* were often growing with duckweed and *Salvinia*. All were labeled as MI by reference to the previously collected *Azolla* from Paraguay and by features of the megasporocarp of 4067.

Allozymes

Seedlings were successfully germinated from spores collected from MI 4067 in the

Chaco plain. Of the 13 loci analyzed in 52 filial plants of MI 4067, 8 were noted to be polymorphic (Table 3a). Heterozygotes were observed in 5 loci of PGI, PGM (2 loci) and SKDH (2 loci) (Table 3b).

Table 3a. Allelomorphic frequencies of polymorphic enzymes in *Azolla microphylla* of Paraguay.

Enzyme/	Gran Chaco (green)[a]				Other Regional (red)[b]				F_1 of MI 4067		
	Allelic Frequencies										
Locus	a	b	c	d	a	b	c	d	a	b	c
AAT-1	0.71	0.22	0.07		0.21	0.65		0.14			
AAT-2	0.14	0.86			1.00						
PGI	0.89	0.11			0.71	0.19			0.99	0.01	
PGM-1	0.29	0.71			0.64	0.36			0.14	0.86	
PGM-2[c]	0.44	0.44		0.11	0.08	0.67	0.25		0.77	0.23	
SKDH-1	0.67		0.33		0.10	0.70	0.20		0.93	0.07	
SKDH-2	0.31	0.44	0.25		1.00				0.53	0.47	
AMY	0.75	0.25			0.50	0.50					
MDH-1	not done				not done				0.02	0.96	0.02
MDH-3	not done				not done				0.73	0.27	
TPI-1	incomplete				incomplete				0.67	0.13	0.20

[a]Accessions 4001, 4003, 4009, 4014 and 4066 - 4071 series

[b]Accessions from 4062 - 4065 and 4072 - 4075 series

[c]Alleles a, b, and d are the fast alleles at locus 2

Table 3b. Observed heterozygosity in offspring from MI 4067.

ENZYME/LOCUS	PGI	PGM-1	PGM-2	SKDH-1	SKDH-2
% HETEROZYGOSITY	0.02	0.08	0.13	0.20	0.06

Cumulative results from all populations, scrutinized for markers separating "green" and "red" *Azolla*, showed similar fingerprints to each other and to accessions labeled MI from elsewhere. But distinctions were observed in several enzymes (Table 3a). *Azolla* of the Gran Chaco possessed electromorphic PGM-2 alleles unlike that of MI from elsewhere in Paraguay (except 4074). These alleles also have not been observed in other New World species (Zimmerman et al., 1989b). Some accessions of red *Azolla* had loci for TPI-3 which differed from that of the other group. No variability was found in red *Azolla* for AMY, AAT-2, and SKDH-2.

A few unique accessions were identified or confirmed from these data (e.g., 4001, 4003). IDH, 6PGD-1, and XDH were not observed to be polymorphic, but the staining intensity of those enzymes were inadequate for interpretation in some accessions. DIAP appeared polymorphic but with poor resolution.

DNA polymorphisms

RAPDs were also pertinent in fingerprinting accessions of *Azolla* but more definitive in depicting subspecific groups. A selection of amplified bands illustrated direct genetic distinctions between the red and green Paraguayan *Azolla*. Examples are shown in Fig. 2 with the CS26 and CS18 primers. The CS26 primer separated red accessions (major DNA band at 400+ bp) from those of the Gran Chaco (major band near 750 bp). Green *Azolla* collected in 1979 or 1988 exhibited the same DNA marker. A distinctive dual-band pattern was observed for a FI control (1537). The hybrid 4028 (MI x FI) could

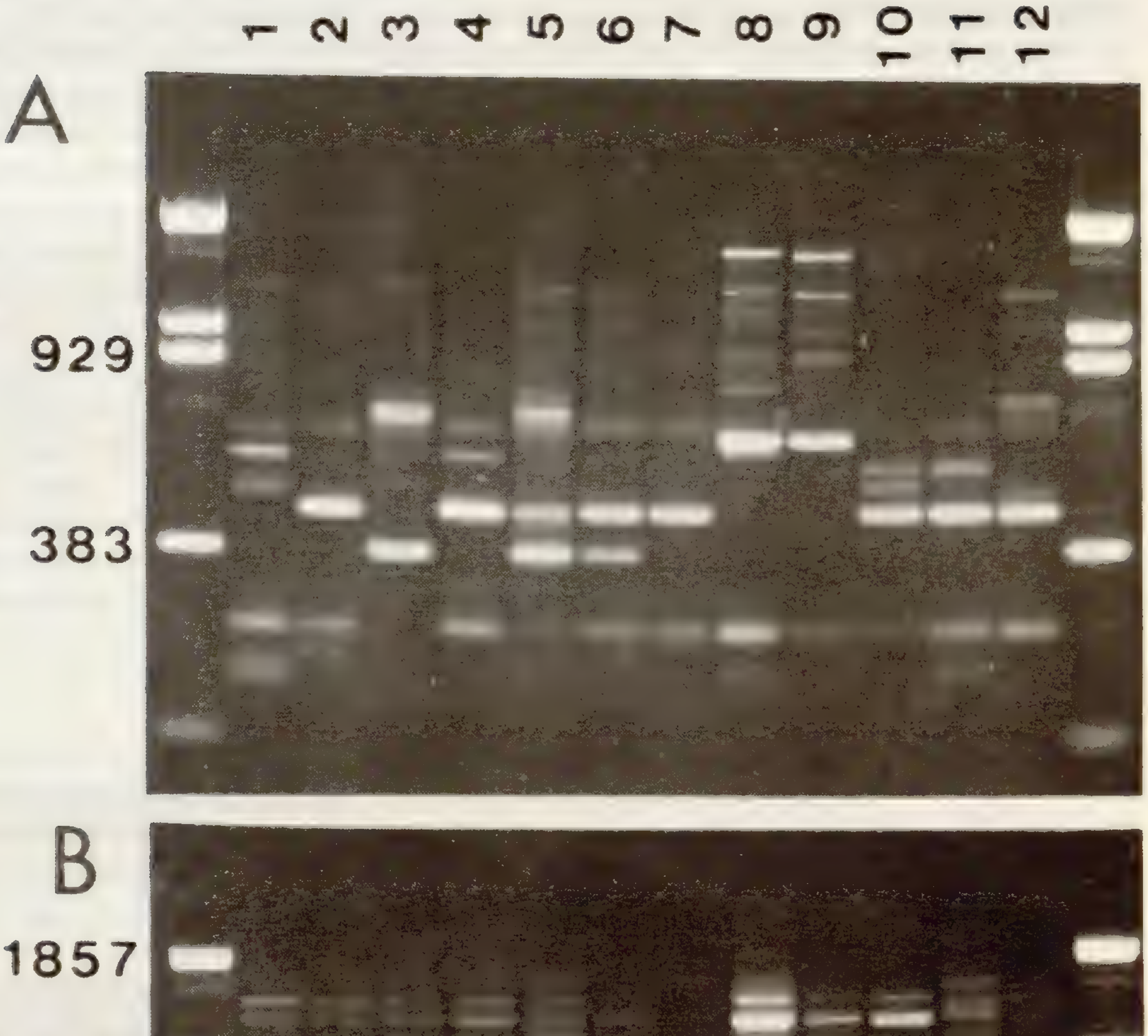

Fig. 2. Random amplified polymorphic DNA (RAPD) patterns for *Azolla microphylla* and one *A. filiculoides* (1537) using primer CS26 (T T G G T C G G A A) (top gel) and CS18 (T T T G G C T C T G). The order of accessions (red *Azolla* clones are underlined), left to right, is:

	1	2	3	4	5	6	7	8	9	10	11	12
a. CS26 –	M2	M3	1537	4014	4028	4031	4052	4062	4064	4067	4068	4069
b. CS18 –	4062	4072	4073	4074	4075	4501	4503	4504	M3	4014	4052	4064

be identified, but not the 4052 rong-ping hybrid. The 4031 accession yielded the marker of the green *Azolla* (and another one equal in size to one FI band). A MI control from the Galapagos Islands (M2) is seen in Fig. 2 to resemble none of the others.

The red *Azolla* of central Paraguay were recognizable by markers such as the major band near 900 bp generated by the CS18 primer (Fig. 2). Two results were repeatedly observed: 1) that two Brazilian accessions, 4501 and 4502, were similar to the red Paraguayan MI, and 2) the green MI accessions yielded similar RAPDs as MI from other regions, so were often not as distinctive as the red MI.

Discussion

The climatic conditions of Paraguay are suitable for *Azolla* growth, but actual occurrence depends upon environmental variables such as soil conditions or microclimate. Plants of this genus grew vigorously in the mostly homogenous soils of the semi-arid Gran Chaco, despite less rainfall and higher temperatures than elsewhere. Soil types of this region are kastanozems and solonetz bearing the same geologic history (FAO/UNESCO, 1968, 1971).

Azolla has a tremendous potential biomass doubling time (<2 days fresh weight) for a vascular plant, given optimal growth conditions. Many of the sampled natural populations may easily have propagated from a single founder, and would be nearly genetically identical. This has been observed elsewhere (Zimmerman, unpublished). Accordingly, the possibility that the collected accessions were clonal representatives of their populations could not be initially dismissed.

Presence of a fully sporulating population proved otherwise. Successful germination of seedlings from spores of the Chaco MI 4067 showed that populations of this heterosporous fern could functionally sporulate in this subtropical ecosystem, and that established populations were not just vegetatively propagated. Genetic recombination was exhibited by the presence of polymorphic loci in the F_1 generation. This report is the first for *Azolla* that includes intrapopulational information.

Azolla samples collected for agronomic purposes are grown continually with periodic thinning until one clonal culture results. Since relative agronimic hardiness does not vary within a population, extensive sampling is not undertaken. Individual genotypes, representing a given locale or geographic area, are then tested for their agricultural potential as biofertilizers.

Likewise, enzyme and DNA fingerprints are realized to find relevant markers of species or desired ecotypic traits. In this study, a genetic basis to separate the two Paraguayan phenotypes was illustrated, with *Azolla* of the central region of Paraguay as the more distinguishable. RAPD identification of hybrid *Azolla* is also apparently feasible.

This study intentionally produced RAPD fingerprints of the intact *Azolla*-symbiont association, so the proportion of DNA contributed from host or symbiont was not a relevant question (cyanobacterial contribution to zymograms is nil; Zimmerman et al., 1989a). Prokaryotic DNA present after *Azolla* DNA isolation by our procedure should be almost non-existent, and prior tests for DNA restriction fragment length polymorphisms with cyanobacterial or chloroplast gene probes did not yield bands attributable to the cyanobiont. Also non-random amplifications, using primer pairs for the trnL (UAA) cpDNA intron and an adjacent spacer region (Taberlet et al., 1991), amplified a unique band using purified cyanobiont DNA as a template. An amplified band of the same size

never appeared from DNA of the intact parental *Azolla* association or other accessions (Zimmerman, unpublished).

Further information concerning the distribution of *Azolla* species in South America is needed. One region for future survey is the Rio Parana system in Paraguay and Argentina; *A. filiculoides* and *A. caroliniana* are reportedly part of this river's flora in northeast Argentina (Bonetto, 1970). Another is the remainder of the Gran Chaco plain for *A. caroliniana* in Argentina and *A. mexicana* in Bolivia (Svenson, 1944). In addition to documenting species ranges, more agroecotypes would be located and saved for future application.

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Lack of Isozyme Variation in the Agamosporous Fern *Dryopteris remota* (A. Braun) Druce

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When we study apomictic taxa two basic questions are of special relevance. The first is the question of their phylogenetic and/or geographic origin, the second is that of their genetic composition (variability or uniformity). There exists a variety of allozyme studies in apomictic angiosperms (Asker & Jerling, 1992; Battjes et al., 1992) which allows insights into the genetic patterns of apomicts and their evolution. In apomictic plants various genetic structures are observed. For example, the populations of apomictic species are sometimes multiclonal and the genetic differences between populations may often be relatively high (Ellstrand & Roose, 1987).

According to Walker (1984) about 10% of all ferns are agamosporous. In the last decade a few investigations on apomictic (agamosporous) ferns using isozyme methods were published. In contrast to the earlier opinion, that agamosporous species are exclusively of hybrid origin (Walker, 1984), it was shown that in some examples, for instance *Pellaea andromedifolia* (Gastony & Gottieb, 1985), the *Pellaea glabella* complex (Gastony, 1988), and in *Dryopteris sparsa* (Darnaedi et al., 1990b), autopolyploidy may play an important role in the formation of apomicts. The apomictic species *Pellaea atropurpurea* (Gastony & Windham, 1989), *Asplenium unilaterale* (Watano & Iwatsuki, 1988), *Pteris cretica* (Suzuki & Iwatsuki, 1990), and *Dryopteris yakusilvicola* (Darnaedi et al., 1990a), on the other hand seem to have originated through hybridization events between different species.

Agamospory in *D. remota* was first described by Fischer (1909). It was Döpp (1932) who worked out the detailed cytological background of the agamospory in this fern. Döpp's findings are valid for most of the agamosporous fern species (Walker, 1979). *Dryopteris remota* is triploid and appears to be strictly agamosporous (Fraser-Jenkins & Reichstein, 1984). This European species is distributed from the eastern Pyrenees to the Caucasus but is everywhere rare. *Dryopteris remota* usually grows as single individuals or forms small stands of a few to sometimes about 50 individuals. The populations are isolated from each other, sometimes by large distances. The largest population known so far was described by Benl & Eschelmüller (1973). About 130 individuals were found in one site (2000 x 300 m^2) in Bavaria. In terms of morphological characteristics, *D. remota* seems to be quite uniform (except for characters most likely due to phenotypic plasticity) (Fraser-Jenkins & Reichstein, 1984).

In our investigation the following questions were of interest: Are there different enzyme genotypes, either within populations or between populations from different parts of the distribution area? Do enzyme data point to a unique, or a multiple origin of the species?

MATERIALS AND METHODS

Our investigation consisted of two parts. Firstly, one group of plants was collected in the private garden of Prof. T. Reichstein at Basel who cultivated *Dryopteris remota* from many different locations for many years. These plants were used to estimate the variation

of enzyme genotypes between populations of different areas. (Table 1). Secondly, to test the enzyme variation within a natural population, we also collected *D. remota* from a population, consisting of about 60 individuals, near Härkingen, Canton Solothurn, Switzerland (Table 1).

Table 1: Origin and number of investigated plants. Voucher specimens TR are deposited in herbarium T. Reichstein, Basel; voucher specimen Z in herbarium Z.

Country	Locality	Number of Plants	Voucher
France	Pyrenees, Bagnères-de-Luchon (1400 m.alt.)	1	TR-3866
France	Dépt. Corrèze, Tulle (-)	2	TR-4057
			TR-4058
France	Jura, Dépt. Doubs, Frasme (-)	1	TR-3883
France	Vosges, Hohwald (-)	1	TR-3833
Switzerland	Canton Solothurn, Hägendorf (690 m.alt.)	32	Z, R. Holderegger
			20.7.1993
Switzerland	Canton Zurich, Hütten (680 m.alt.)	1	TR-288
Switzerland	Canton Zurich, Sternenberg (760 m.alt.)	1	TR-3921
Switzerland	Canton St. Gallen, Quarten (950 m.alt.)	4	TR-3858
			TR-3862
			TR-3863
			TR-3864
Switzerland	Canton St. Gallen, Murg (750 m.alt.)	2	TR-3860
			TR-3870
Germany	Black Forest, Wehr (650 m.alt.)	1	TR-1979
Germany	Black Forest, Baden-Baden (near the locus classicus) (-)	1	TR-3894
Austria	Tyrol, Rattenberg (620 m.alt.)	1	TR-4827
Italy	Prov. Varese, Ponte Tresa (270 m.alt.)	1	TR-7329
Croatia	near Zagreb (-)	1	TR-6276
Romania	Carpathian Mountains, Petroseni (1100 m.alt.)	1	TR-3906
Turkey	Prov. Rize, Ardesen (-)	1	TR-4011
Gruzinian Republic	Caucasus, near Alagier and Gizel (-)	1	TR-4031

Starch as well as agarose gel electrophoresis was applied following the methods described by Wendel & Weeden (1989) and Schneller & Scheffrahn (1988). Grinding buffer (for tissues low in phenolics), gel and electrode buffers (No. 1 and No. 5), and staining schedules described by Soltis et al. (1983) were used. The following enzyme systems were tested: GPI, MDH, PGM, SKD, IDH, TPI, G6PD, PGD, GDH, FBA, G3PD. ADH and LAP were not interpretable.

Results

No differences in enzyme phenotypes could be detected neither among the plants of the Härkingen population nor among the various collections of Reichstein. All investigated plants share exactly the same enzyme genotype!

Fig. 1 depicts the zymograms for the different enzyme systems observed by us. Fig. 2 is a photograph of the enzyme phenotype found for PGM. Because of the complexity of the banding patterns of many enzymes, an interpretation of the observed phenotypes on an allelic level following Weeden & Wendel (1989) was not attempted.

Discussion

It has been shown, for many examples of apomictic flowering plants, that genetic variation is a common phenomenon (Asker & Jerling, 1992). Such variation is thought to be mainly due to occasional sexual processes and to the fact that sometimes reduced

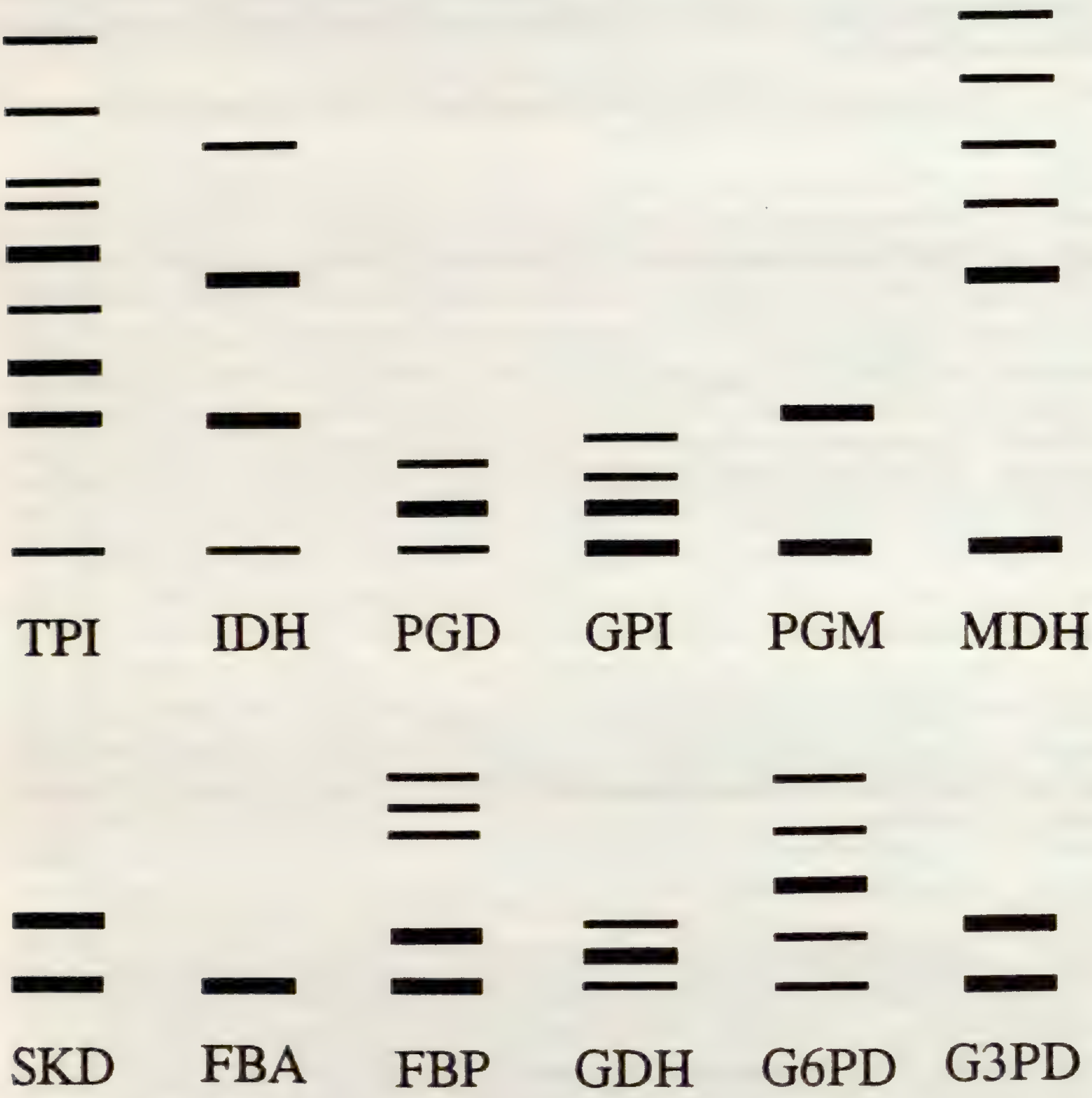

Fig. 1. Zymograms of the different enzyme systems tested in *Dryopteris remota*. All investigated individuals (Table 1) showed exactly the same enzyme phenotype.

gametes are formed. Even within populations the presence of a variety of different genotypes (enzyme phenotypes) can be shown, e.g. in *Taraxacum* (Battjes et al., 1992).

In contrast to this, apomictic fern species lack sexual processes. Nevertheless, apomictic prothalli may form male gametes which can possibly unite with female gametes of sexual relatives and thus participate in sexual processes. Therefore one would expect only a small amount of genetic variability or even none within populations of apomictic fern species. The role of subsexual processes (homoeologous pairing) is still controversial (Klekowski, 1973; Haufler, 1987). If genetic variability exists between or within populations either a multiple origin of the apomictic species, somatic mutations or gene silencing (Werth et al., 1985) must be assumed.

Apomictic fern species may be formed by autopolyploidy (Gastony & Gottlieb,

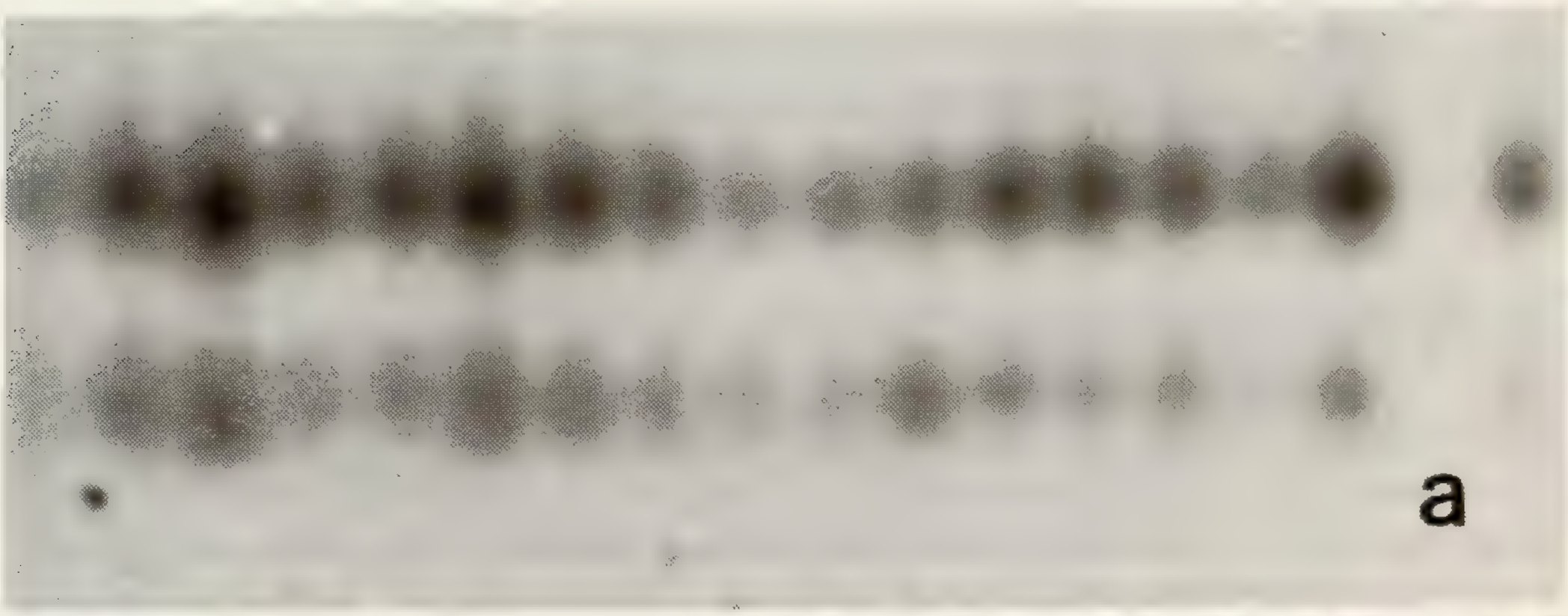

Fig. 2. PGM banding pattern of *Dryopteris remota*. One enzyme phenotype of each location presented in Table 1 is shown; a = lane of marker plant.

1985; Gastony, 1988; Darnaedi et al., 1990b) or allopolyploidy (Gastony & Windham, 1989; Darnaedi et al., 1990a; Suzuki & Iwatsuki, 1990, Watano & Iwatsuki, 1988). Except for *Dryopteris yakusilvicola* (Darnaedi et al., 1990a), which is found only in a comparatively small area, all apomictic fern species so far investigated electrophoretically showed different genotypes between and sometimes even within populations. This would suggest a multiple origin of these apomicts, the occurrence of subsexual processes, or gene silencing.

Our result is in contrast to these findings. There are three ways to interpret the complete lack of enzyme variation in *D. remota:* First, one might argue that even the parents of triploid *D. remota,* which is of hybrid origin, are or were genetically uniform. Second, the selected enzyme data are not representative for the total genome. Genetic variation may therefore exist in other loci (Hamrick, 1982). Third, *Dryopteris remota* has a unique perhaps very recent origin (possibly somewhere in the Caucasian or Mediterranean region) and then has been dispersed by spores over its present area as suggested also by Fraser Jenkins & Reichstein (1984). A solitary origin of an apomictic species was also discussed for *Dryopteris yakusilvicola* (Darnaedi et al., 1990a).

Up to the present it has not been possible to synthesize the triploid *D. remota* by crossing the putative parents, thought to be *D. expansa* and diploid *D. affinis* respectively (Manton 1950); furthermore, phloroglucine patterns do not support this hypothesis (Widén et al., 1976). According to Peroni et al. (1991), diploid *Dryopteris raddeana* (Fomin) Fomin or *D. pallida* (Bory) Fomin subsp. *pallida* and diploid *D. affinis* might be the parent species. Up to now nothing is known about the electrophoretic profiles of these four first named species in Europe. Further investigations using isozymes and in addition to that cpDNA or RAPD may help to solve this problem.

Acknowledgments

We thank Prof. Dr. T. Reichstein for his encouragement and for his help in collecting *D. remota* from quite different locations growing in his garden. We thank Prof. K.U. Kramer, two anonymous reviewers, and Leylâ Yasargil for reading and commenting on our paper.

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AMERICAN FERN JOURNAL: 84(3):99-101(1994)

A New *Selaginella* (Selaginellaceae) From Queretaro and Veracruz, Mexico

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Mexico with its ca. 76 species of *Selaginella* has the third highest number of species of any country in the New World. During the course of my preliminary studies aimed to prepare the treatment of the family Selaginellaceae for the Pteridoflora of Mexico Project (Mikel, Smith, Palacios-Rios, and collaborators, in prep.), a new species was uncovered among a loan of *Selaginella* specimens from Veracruz sent to me by M. en C. Mónica Palacios-Rios (XAL). Later, Bióls. Horalia Díaz Barriga (IEB) provided me with additional material of the new species from Querétaro. Since this novelty is to be included by Bióls. Díaz Barriga (pers. comm.) in her treatment of the genus *Selaginella* for Flora del Bajío, a formal description is required, which I provide below. Further additions to Mexican *Selaginella* are foreseen as ongoing studies by the author continue.

Selaginella (subg. *Stachygynandrum)* arsiclada Valdespino, sp. nov. (Fig. 1A–F)

TYPE: MEXICO: Querétaro: Landa, Tangojo cerca del Río Moctezuma, 300 m, 4 Mar 1988, *H. Díaz Barriga 4627* (Holotype: NY; Isotype: IEB-n.v., PMA).

A *S. reflexa* Underwood et *S. schiedei* A. Braun foliis intermediis apice late acutis, marginibus basin versus breviter ciliatis vel serratis, apicem versus serratis diversa. A *S. reflexa* foliis axillaribus in caule principali eis lateralibus similibus, et a *S. schiedei* foliis lateralibus apice gradatim decrescentibus diversa.

Etymology – Gr., *arsis,* a raising or lifting, and *klados,* branch, twig, stem; referring to the tendency of the apex of the branches to become ascending or to raise up when dry.

Plants epipetric or terrestrial. **Stem** creeping, chlorophyllous to straw-colored, 2–12 cm long, 0.03-0.5 mm diam., not articulate, 2- or 3-branched. **Rhizophores** axillary, borne throughout the stem, 0.18–0.3 mm diam. **Leaves** dimorphic throughout, chartaceous, the upper surface green or yellowish to brown when old, the lower surface green, usually with the acroscopic half whitish (specially in younger leaves), or becoming brown in old leaves. **Lateral leaves** distant on main stem, imbricate at apical region of the stem and on branches, ascending to perpendicular to the stem, broadly ovate to ovate-deltate on main stem, ovate-oblong to oblong on branches, 1–1.4 x 0.5–0.9 mm; base semicordate, the acroscopic base rounded, strongly overlapping the stem, the basiscopic base rounded, not overlapping the stem and usually with a tuft of hairs (the hairs made of two cells, the basal on bulbous, the distal one elongate); margins whitish in a band 1 (2) cells wide with cells elongate and papillate parallel to margin, the acroscopic margin short-ciliate at proximal 1/2 to 1/3, serrate apically, the basiscopic margin short- to long-ciliate at base, serrate apically; apex obtuse, dentate at tip; upper (adaxial) surface primarily made up of rounded cells with irregular to slightly sinuate walls (sometimes with many papillae) on the basiscopic half of the leaves, and near apical portion of the acroscopic half of the leaves, otherwise made up of elongate cells with sinuate walls on the acroscopic half, stomata throughout the surface; lower (abaxial) surface made up of elon-

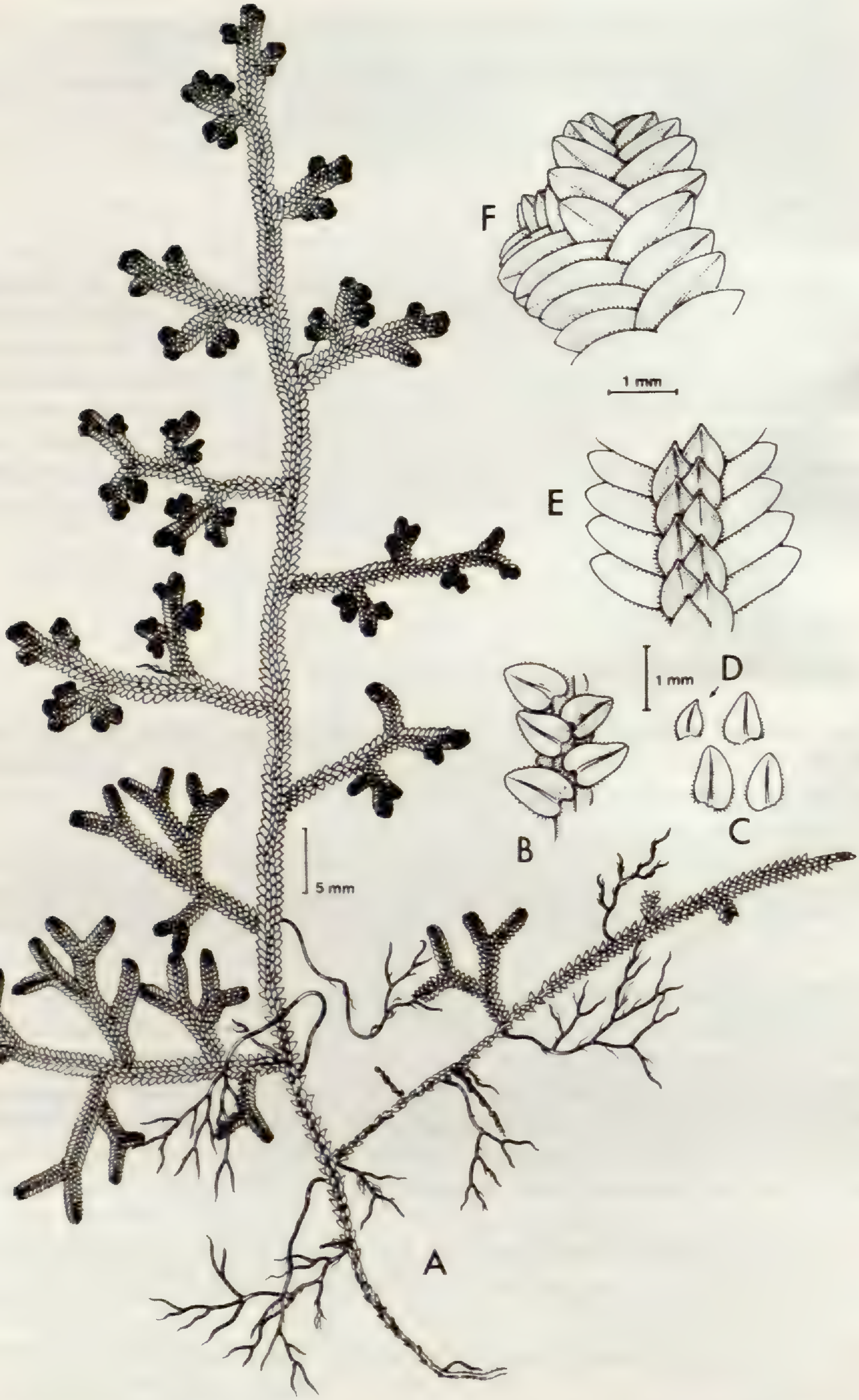

Fig. 1. *Selaginella arsiclada* (drawn by Edmundo Saavedra: all from *Díaz Barriga 4627*, NY). A. Habit. B. Detail of the lower surface of the main stem showing lateral leaves. C. Lateral leaves. D. Median leaf. E. Detail of the upper surface of the main stem showing median leaves. F. Detail of the lower surface of a branch tip.

gate cells with two rows of papillae (more so on acroscopic half of the leaves) and with mostly straight walls, stomata restricted to midvein. *Median leaves* imbricate, ascending, ovate, 0.6–0.8 x 0.38–0.55 mm; base oblique to slightly cordate, the outer base frequently with a tuft of hairs; margins whitish in a band 3 or 4 cells wide with cells elongate and papillate parallel to margin, short-ciliate basally, serrate apically; apex broadly acute, dentate at tip; upper (abaxial) surface made up of rounded and papillate cells with irregular to slightly sinuate walls, stomata restricted to midvein; lower (adaxial) surface made up of elongate cells without papillae and with sinuate walls, stomata throughout surface. **Axillary leaves** similar to lateral ones, except the base more obviously cordate, or oblong on branches. **Strobili** terminal, quadrangular, 2–5 mm long. **Sporophylls** monomorphic, ovate, 0.9–1.2 x 0.5–0.6 mm; base rounded; margins hyaline, short-ciliate near base, serrate apically, the cilia and teeth ascending. **Megasporangia** in two ventral rows; **microsporangia** in two dorsal rows. **Megaspores** white, seemingly smooth, 200–240 µm diam.; **microspores** orange, capitate on distal face, 21–28 µm diam.

Habitat and distribution: on dry clay at river banks in seasonally dry area, or soil and rocks in tropical "subperennifolio" forest; ± 200–300 m; known only from Querétaro and Veracruz, Mexico.

PARATYPES: MEXICO: Querétaro: Landa, Tangojo cerca del Río Moctezuma, 300 m, 4 Mar 1988, *Díaz Barriga 4267* (IEB-n.v., NY). Veracruz: Palmillo, Dec 1919, *Purpus 8463* (mixed coll. *8463b* at MO). Xalapa: road from Xalapa to Veracruz, Puente Nacional, down river from Resort Hotel, [± 200 m], 1 Jan 1980, *Sperling 4049* (XAL).

Selaginella arsiclada does not seem to have a close relative among other Mexican *Selaginella*. It may be confused, however, with *S. reflexa* Underwood and *S. schiedeana* A. Braun, because of its creeping habit, the axillary rhizophores that are borne throughout the stem, its ovate median leaves, and its whitish leaf margins. *Selaginella arsiclada* can be chiefly distinguished from these two species by its median leaves with a broadly acute (vs. long-acuminate to aristate) apex, the margins shortly ciliate or serrate basally, and serrate apically (not long- to short-ciliate throughout). It differs further from *S. reflexa* in the axillary leaves on the main stem being similar to the lateral leaves (vs. obovate in *S. reflexa)*, and from *S. schiedeana* by its lateral leaves with the apex gradually tapering (vs. almost equal in width to the base in *S. schiedeana*).

ACKNOWLEDGMENTS

Thanks are due to M. en C. Mónica Palacios-Rios for logistical help, and to Dr. Victor Rico G., both of whom made attempts to collect additional specimens from Veracruz of the species, Bíol. Horalia Díaz Barriga for sending me additional specimens, Dr. Rupert Barneby for his help with the Latin diagnosis, Mr. Edmundo Saavedra for rendering the illustration, and Dr. John T. Mickel for his support and advice during my studies on the genus *Selaginella*. This paper is part of a series of contributions leading to the publication of the Pteridoflora of Mexico (Mickel, Smith, Palacios-Rios, and collaborators, in prep.).

Shorter Notes

A new name in Diplazium. — Eight new taxa were described in a treatment of the genus *Diplazium,* recently published in the "Flora of Ecuador." It has been brought to my attention by David Lellinger (*in litt.*) that one of these is a later homonym of *D. ciliatum* Price (Contr. Univ. Michigan Herb. 17:20. 1990), a fern which occurs in the Philippines. Consequently, a new name is required for the Ecuadorean species, the nomenclature for which is given here.

Diplazium navarretei Stolze, *nom. nov.* for *D. ciliatum* Stolze, and with the same type. *Diplazium ciliatum* Stolze, in Flora of Ecuador (eds. Harling & Andersson) 49:45. 1994, not *D. ciliatum* Price, 1990.

The species is named for Hugo Navarrete, pteridology student at the Departamento de Biologia, Pontificia Universidad Catolica del Ecuador, in Quito. During my numerous field trips throughout Ecuador in 1992 Sr. Navarrete acted alternately as collecting assistant, guide, chauffeur, cook, and close companion. It is in recognition of his friendship and invaluable assistance that the new name is assigned to this species. – R. G. STOLZE, 912 PIRATE COVE LN., VERO BEACH, FL 32963.

***Psilotum nudum* New to North Carolina.** — The unusual pteridophyte, *Psilotum nudum* (L.) P. Beauv. (Psilotaceae) is described as being pantropical with populations extending northward through Florida and southern Georgia to northeastern South Carolina and westward in northern Louisiana and eastern Texas with a disjunct population in Arizona. Imagine our surprise and delight when on December 21, 1992, one of us (JPP) discovered *Psilotum nudum* in a swamp in Chowan County, North Carolina. Water tupelo *(Nyssa aquatica)* and Bald cypress *(Taxodium distichum)* dominate this swamp located approximately 1 km ESE of Harris Landing, North Carolina, which is on the east bank of the Chowan River, approximately 700 meters south of a point on State Road 1210, approximately 500 meters west of the junction of S.R. 1210 and S.R. 1211. The site is included on the Valhallah, North Carolina, Quadrangle U.S. Geological Survey 7.5 minute topographic map. This population represents a northeastward range extension of approximately 360 km, is a state record, and is likely the northernmost occurrence of *Psilotum* in the United States.

The *Psilotum* colony consists of about a dozen small but healthy looking stems which branched dichotomously (for the most part) one to five times. Although all individuals were primarily dichotomously branched there were some instances in which a branch divided into three stems instead of the usual two. The above ground stems measured around 5–10cm and all were sterile with no evidence of sporangia having been formed. Most stems emerged singly from the ground at least 15cm or so from the next nearest stem. The main body of the colony was in an area about 20m in circumference with one plant about 150m or more from the main colony. On January 18, 1993, three small portions of above ground branches were collected to serve as vouchers and have been deposited as follows: Collection No. JP-93-4, NCU; Collection No. JP-93-5, MICH; Collection No. JP-93-6, US.

Psilotum plants were growing terrestrially on a slightly elevated strip of land composed of Seabrook sand which grades into Dorovan muck in the swamp proper. The plants in main colony were located in the lowest region of this strip of projecting highland near where it is divided by a short, flowing creek. All of the *Psilotum* plants grew either at the base of trees where the soil was slightly elevated or on small mounds of soil

caused originally by the tipping up of the root system of a wind-thrown tree. A soil sample from this area had a pH of 5.6. The one *Psilotum* plant not growing in the main colony was growing in a higher area with much better drainage at the base of the dead stump of a large American beech tree *(Fagus grandifolia)* which had recently died and fallen over. This plant, on December 21, 1993, consisted of two closely spaced aerial stems, one of which had recently turned soft brown and appeared dead. On 1 January 19, 1993, neither stem could be found despite a careful search.

Associates of the *Psilotum* colony included *Acer rubrum, Ulmus americana, Fagus grandifolia, Liriodendron tulipifera, Ilex opaca, Nyssa sylvatica var. biflora, Taxodium distichum, Carpinus caroliniana, Berchemia scandens,* and *Leucothue axillaris.*

Psilotum nudum records were sought from North Carolina: CLEMS, DUKE, GA, MICH, MO, NCU, NCSC, NY, ODU, TENN, UNCC, US, USCH, WM, WNC, VPI and Longwood College. No specimens with localities north of South Carolina were located.

Suspecting that *Psilotum* so far north might be adventive, it seemed advisable to check at two greenhouse complexes, one approximately 3 km northeast of the site and the other approximately 3 km east of the site. These greenhouses were all owned by an extended family named Leary. Specimens were shown to nearly all of the proprietors including the octagenerian founder of the greenhouse business in the area. All of the greenhouse men were completely unfamiliar with *Psilotum* and said that they had never grown it or seen it although they were informed that it sometimes comes up as a weed in greenhouse pots. Even so, it may have grown unnoticed in their greenhouse and escaped.

Whether adventive or natural, the *Psilotum* population shares several characteristics with other populations reported at the northern extremities of its range. The Chowan County plants are all very small, sterile, and terrestrial rather than epiphytic. This is consistent with reports from the literature of *Psilotum* populations in South Carolina (Swails, Amer. Fern J. 63:8, 1973), Alabama (Freeman, Amer. J. 77:102–105), Louisiana (Reese, Amer. Fern J. 50:269–270, 1960), and Texas (Lodwick, Amer. Fern J. 65:62, 1975) which were all noted as being small (12 cm maximum), sterile, and terrestrial. This also tallies with a small colony of *Psilotum* extending approximately four feet along the east side of a building under the shade of a crepe myrtle tree on the North Carolina State University campus in Raleigh, North Carolina. These plants are also small (10 cm maximum), sterile, and terrestrial and have been so for four or five years (J.S. Hardin, personal communication). Hardin suspects that these plants were introduced in a load of mulch from the deep south.

It seems probable that the growing season is too short in these areas for spores to form. Likewise, the winters may be too cold for a rootstock to survive epiphytically. J.P. Freeman (personal communication) noted that *Psilotum* he dug from east central Alabama and grew in a greenhouse took 2 1/2 years to produce spores and have been fertile since. He also noted these plants were indistinguishable from *Psilotum* of tropical origin. Thus it could be that these northern *Psilotum* plants are a result of a rain of spores from more tropical regions as suggested by W. H. Wagner, Jr. (personal communication).

The exciting find of *Psilotum* in Chowan County and in Raleigh, North Carolina, warrants further research into its northern distribution and poses questions about its life history in the region. There are abundant swamp areas similar to the Chowan County site all along the coastal plain and a careful exploration may reveal *Psilotum* in the vicinity of the Dismal Swamp in southeast Virginia. The Alabama population reported by Freeman

(loc. cit.), as well as the one in Raleigh, is growing on upland clay-based soil which would warrant searching the outer piedmont of North and South Carolina.

The authors plan to monitor the population structure, reproductive mode, effects of microhabitat manipulations of the known *Psilotum* in North Carolina, and will continue to seek new populations which may exist in the most northern part of its range. — JESSE P. PERRY III, N.C. State Museum of Natural Sciences, P.O. Box 29555, Raleigh, NC 27626 and LYTTON J. MUSSELMAN, Department of Biological Sciences, Old Dominion University, Norfolk, VA 23529-0266.

Review

Ferns for American Gardens, by John T. Mickel, 1994. Macmillan Publishing Co., New York. ISBN 0-02-584491-1. $60.00 US, hardback. 370 pp.

Most garden books provide little information on ferns, a serious fault. John Mickel has changed all that with his newest work. His book includes chapters on fern structure, growing and gardening with ferns, propagating ferns, and hardy ferns for American gardens. The latter chapter, being most of the book, features discussions of over 400 species (with their many subspecies, varieties, cultivars, and sports) with 364 full color photographs. The ferns are arranged alphabetically by genus. The frond form, plant habit, availability, hardiness, and ease of cultivation are provided along with notes and commentary on its biology or relations. The major types of "sports", such as undulate, ramose, congested, cruciate, and crested are discussed along with historical notes on the Fern Craze era of "pteridomania" when ferns were decoration motifs. The writing style allowed for the author's well known enthusiasms and humor. Fern partners that grow well with ferns and enhance the presentation of ferns in the landscape are listed. Access to other literature is provided by lists of state fern floras, general fern references, and generic monographs. The book jacket claims that this is "The definitive guide to selecting and growing more than 500 kinds of hardy ferns", and so it is. We have needed this book for a long time; it belongs on every pteridologist's and gardener's shelf of essential books. — JAMES H. PECK, University of Arkansas at Little Rock, Little Rock, AR 72204.

INFORMATION FOR AUTHORS

Authors are encouraged to submit manuscripts pertinent to pteridology for publication in the *American Fern Journal.* Manuscripts should be sent to the Editor. Acceptance of papers for publication depends on merit as judged by two or more referees. Authors are encouraged to contribute toward publishing costs; however, the payment or non-payment of page charges will affect neither the acceptability of manuscripts nor the date of publication.

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Papers longer than 32 printed pages may be sent to the Editor of *Pteridologia* (Memoir Editor, see cover 2)

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Gifts and bequests to the Society enable it to expand its services to members and to others interested in ferns. Back issues of the Journal and cash or other gifts are always welcomed and are tax-deductible. Inquiries should be addressed to the Secretary.

Phylogeny, Biogeography, and Population Biology of *Osmunda* Species: Insights From Isozymes

JIANWEI LI AND CHRISTOPHER H. HAUFLER
Department of Botany, University of Kansas, Lawrence, Kansas 66045

Osmunda is intermediate between the eusporangiate and leptosporangiate ferns in many aspects and therefore has a special systematic position among extant pteridophytes. It is this position, together with the small number of extant *Osmunda* species and relatively extensive fossil record, that has generated great curiosity about this genus. Not only have considerable morphological comparisons been developed (Hewitson, 1962; Miller, 1967, 1971), but also aspects of palynology (Hanks & Fairbrothers, 1981), serology (Peterson & Fairbrothers, 1971), and DNA structure (Stein & Thompson, 1975; Stein et al., 1979; Stein et al., 1986) have been explored. Most of this work has focused on three extant *Osmunda* species: *O. regalis, O. claytoniana* and *O. cinnamomea,* which are native to North America. Because of their distinctive morphological differences, these three species can be readily delimited, but their phylogenetic relationships are obscure. For about a century, pteridologists have been debating the evolutionary history of these three species and currently there are three competing hypotheses: (1) *O. claytoniana* and *O. regalis* are more closely related to each other than either of them is to *O. cinnamomea* (Faull, 1901; Miller, 1967, 1971; Wagner et al., 1978), (2) *O. claytoniana* has more in common with *O. cinnamomea* than with *O. regalis* (Hewitson, 1962; Peterson & Fairbrothers, 1971; Stein & Thompson, 1975; Hanks & Fairbrothers, 1981), and (3) all three species arose from a common ancestor at about the same time (Stein et al., 1979; Stein et al., 1986).

Table 1. Locations and population designations of 20 populations of *Osmunda* used in allozyme analysis. N = population size.

Species	Pop.	Locations	N
O. regalis	a	Chautauqua Co. Kansas	48
	b	Saline Co. Arkansas	26
	c	Pulaski Co. Arkansas	57
	d	Somerset Co. United Kingdom	19
	e	Somerset Co. United Kingdom	3
O. cinnamomea	f	Saline Co. Arkansas	30
	g	Pope Co. Arkansas	31
	h	Pulaski Co. Arkansas	48
	i	Pembroke Co. Virginia	31
	j	Pembroke Co. Virginia	27
	k	Pembroke Co. Virginia	24
	l	Sullivan Co. New Hampshire	47
	m	Sullivan Co. New Hampshire	4
O. claytoniana	n	Pope Co. Arkansas	19
	o	Pembroke Co. Virginia	17
	p	Pembroke Co. Virginia	4
	q	Sullivan Co. New Hampshire	27
	r	Sullivan Co. New Hampshire	22
	s	Sullivan Co. New Hampshire	19
O. xruggii	t	Craig Co. Virginia	3

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Table 2. Allele frequencies in 19 populations of *Osmunda*

Loci/Alleles		Populations																		
		a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s
GPI-1	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.842	0.375	0.517	0.454	0.538
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	3	0.408	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.176	0.375	0.483	0.432	0.463
	4	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.938	1.000	1.000	0.938	1.000	0.000	0.000	0.000	0.250	0.000	0.000	0.000
	5	0.592	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.023	0.000
	6	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GPI-2	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGM-1	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.089	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.822	1.000	0.911	0.917	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000
	3	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.178	0.000	0.000	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
PGM-2	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.159	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	3	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	0.904	0.917	0.841	1.000	0.000	0.000	0.000	0.000	0.000	0.000
	4	0.563	0.885	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	5	0.000	0.077	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.096	0.063	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000
	6	0.438	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TPI-1	1	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
TPI-2	1	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	1.000	1.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000
LAP	1	0.061	0.365	0.510	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.724	0.462	0.088	0.000	0.000	0.000	0.500	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000
	3	0.214	0.173	0.402	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000
	4	0.000	0.000	0.000	0.000	0.000	1.000	0.500	1.000	1.000	0.714	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000
	5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.286	0.813	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	6	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.188	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SkDH	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.043	0.444	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.804	0.556	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000
	3	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.152	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000
	4	0.739	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000
	5	0.239	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	6	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	7	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
HK	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.149	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.071	0.040	0.000	0.639	0.375	0.452	0.683	0.372	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	3	0.888	0.860	1.000	0.000	0.000	0.548	0.300	0.436	0.850	0.554	0.646	1.000	0.750	0.881	1.000	1.000	1.000	0.818	1.000
	4	0.041	0.100	0.000	0.361	0.625	0.000	0.017	0.043	0.150	0.429	0.354	0.000	0.250	0.095	0.000	0.000	0.000	0.182	0.000
	5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000
ALD	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	0.852	1.000	1.000
	3	0.939	1.000	1.000	0.722	0.750	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.037	0.000	0.000
	4	0.061	0.000	0.000	0.278	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.111	0.000	0.000
MDH-1	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	0.867	0.875	0.875
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	3	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.133	0.125	0.143
	4	1.000	1.000	1.000	1.000	0.750	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000
	5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	6	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-2	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000
	3	1.000	1.000	1.000	1.000	0.750	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000
	4	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	6	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000
MDH-3	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.469	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.909	0.704	0.563	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.531	1.000	0.000	0.000	0.000	0.000	0.000	0.000
	4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.091	0.296	0.438	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	5	0.000	0.000	0.000	0.000	0.000	0.806	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	6	1.000	1.000	1.000	0.500	0.500	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000
	7	0.000	0.000	0.000	0.000	0.000	0.194	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	8	0.000	0.000	0.000	0.500	0.500	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	1.000	1.000

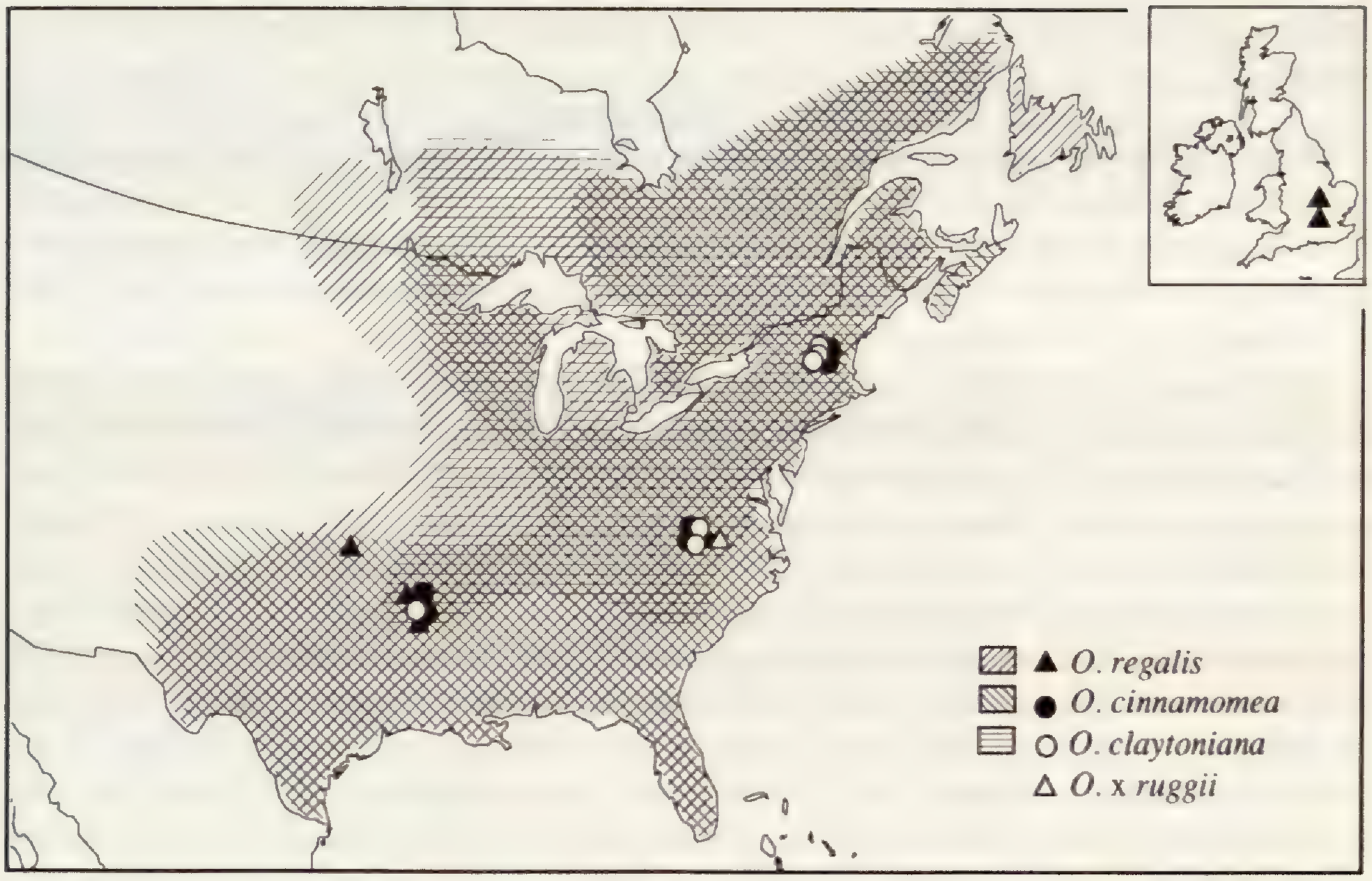


Fig. 1. Distributions (shading) of three *Osmunda* species native to North America and locations (symbols) of the populations surveyed isozymically in North America and Great Britain (inset).

Osmunda is cosmopolitan and widely distributed in tropical and temperate regions. In North America, *Osmunda regalis* ranges from the southeastern Canadian provinces to Florida, and west to Texas and New Mexico (Fig. 1), *O. claytoniana* is less widely distributed and is not found in the southern United States, and *O. cinnamomea* occurs over most of the eastern half of North America. Obviously, these three species have great regions of sympatry. However, even though the three species are often living side by side, only a few hybrids derived from secondary contact have been discovered. One hybrid, named *O. xruggii,* hypothesized to have resulted from crossing between *O. regalis* and *O. claytoniana,* was found once as a wild population in Craig County, Virginia (Tryon, 1940; Wagner et al., 1978). The rarity of hybrid plants suggested that the genetic distance among the three species should be large and that the species had developed effective mechanisms of reproductive isolation during their long evolutionary history.

Electrophoretic analysis of isozymes has been proven to be a powerful technique for exploring genetic variability and breeding systems (Brown, 1979; Gottlieb, 1981; Hamrick, 1982; Crawford, 1990), as well as evolutionary patterns and phylogenetic relationships in seed plants (Crawford, 1983, 1985, 1990). This technique has also been applied to similar studies of pteridophytes successfully (Paris & Windham, 1988; Werth et al., 1985; Soltis & Soltis, 1986, 1988; Haufler, 1985a, 1985b; Gastony, 1988; Pryer & Haufler, 1993). Isozyme data have led to new insights about the patterns and processes of evolution in homosporous ferns.

Because we have little knowledge about the population biology of *Osmunda* species, in this study we applied isozymic electrophoresis to 1) examine genetic variability and breeding systems of the populations, 2) test the hypothesis that there is a great genetic distance among the species, and 3) attempt to assess their phylogenetic relationships. In

addition, the isozymic data were used to assess the proposed parentage of the hybrid, *O.* ×*ruggii*.

Materials and Methods

Fresh sporophyte leaves of the three *Osmunda* species and a putative interspecific hybrid were collected from natural populations (Fig. 1 and Table 1). With the exception of four small populations (<5 individuals in populations **e, m, p,** and **t**), the remaining 16 populations averaged 31 individuals. A total of 506 individuals from these 20 populations were investigated electrophoretically.

Fresh leaves were ground in cold phosphate-PVP extracting buffer (Soltis et al., 1983) and the homogenate was absorbed into paper wicks. The saturated wicks were stored at -80°C until subjected to gel electrophoresis. Because the resolution of the bands for various enzyme systems differs depending on the gel and electrode buffers used, we tested the *Osmunda* species in a variety of buffer conditions. The clearest band patterns of the investigated isozymes were obtained using the following electrode buffers: glucose phosphate isomerase (GPI), phosphoglucomutase (PGM) and triosephosphate isomerase (TPI) were run in system 6 of Soltis et al. (1983). Buffer system 8 (Haufler, 1985a) was used for hexokinase (HK) and leucine aminopeptidase (LAP) and system 11 of Soltis et al. (1983) was used for aldolase (ALD). For malate dehydrogenase (MDH) and shikimic dehydrogenase (SkDH), the morpholine system of Werth (1985) was used. A liquid staining method was employed for LAP while the other seven isozymes followed agarose-overlay stain protocols (Soltis et al., 1983).

Based on the standard quaternary structure and cellular compartmentalization reported for plant enzyme systems (Kephart, 1990), the eight enzymes from our *Osmunda* specimens were interpreted to be coded by 13 loci (Table 2). In order to ascertain the number of alleles for each locus, representatives of the 20 populations were placed side by side on the same gel. For isozymes having more than one locus, the most cathodal locus was assigned the number 1, and the more anodal loci were numbered consecutively.

Once the genotypes of all individuals had been determined, the data were analyzed using BIOSYS-1 (Swofford & Selander, 1981). Gene frequencies in each population, average number of alleles per locus, percent of loci polymorphic, heterozygosity estimates, and genetic identity and distance at the population level were calculated. To assess the genetic variation within the populations and genetic distances among the three species, mean values were calculated from all the populations.

Results

Of the 13 loci analyzed, only one, GPI-2, was invariant both intraspecifically and interspecifically; the other 12 loci varied to different degrees either within species or among species (Table 2). PGM-1 was monomorphic among the populations of *O. regalis* and *O. claytoniana*; however, these two species did not share the same allele at this locus. The locus showing the greatest variation was MDH-3, where eight alleles were detected. To summarize genetic variability, the average number of alleles per locus (A), percent loci polymorphic (P) and heterozygosity estimates for 19 populations (H) are shown in Tables 3 and 4. Among the five populations of *O. regalis,* four isozymes, TPI-1, TPI-2, GPI-2 and PGM-1, were monomorphic and the other loci were polymorphic with percentages between 7.7 and 46.2. Eight populations of *O. cinnamomea* possessed three monomorphic loci, GPI-2, TPI-1, and ALD, and other enzyme loci varied with 7.7 to 46.2% of loci polymorphic. TPI-2 did not show band expression in gels from the popula-

tions of *O. cinnamomea*. The six populations of *O. claytoniana* had relatively low levels of polymorphic loci (7.7-23.1%), and four monomorphic loci were detected (GPI-2, PGM-1, PGM-2, and TPI-1). Because of these low levels of polymorphic loci, *O. claytoniana* had the lowest average genetic variability among the three species (Table 4).

Table 3: Genetic variability at 13 loci in 19 populations of *Osmunda*. A: mean number of alleles per locus; P: proportion of loci polymorphic; H_{ob}: observed proportion of loci heterozygous; H_{exp}: expected proportion of loci heterozygous.

Pop.	A	P	H_{ob}	H_{exp}
a	1.8	0.462	0.073	0.166
b	1.7	0.231	0.059	0.099
c	1.2	0.077	0.016	0.046
d	1.3	0.231	0.111	0.112
e	1.4	0.385	0.096	0.184
f	1.5	0.154	0.082	0.078
g	1.3	0.154	0.128	0.081
h	1.7	0.385	0.042	0.162
i	1.4	0.231	0.067	0.081
j	1.5	0.385	0.087	0.134
k	1.6	0.462	0.077	0.132
l	1.3	0.154	0.062	0.069
m	1.1	0.077	0.000	0.033
n	1.2	0.154	0.095	0.056
o	1.1	0.077	0.018	0.023
p	1.2	0.077	0.058	0.058
q	1.4	0.231	0.055	0.080
r	1.5	0.231	0.072	0.092
s	1.2	0.154	0.055	0.065

The unweighted pair-group method was used to determine the genetic identities and distances among the 19 populations (Table 5) and the results indicated that there were smaller genetic distances among the conspecific populations than among interspecific populations. Genetic distances correlated with geographic distribution of the populations; in most cases, populations that had a greater geographic distance also had a greater genetic distance. Average interspecific genetic identities and distances are listed in Table 6. *O. regalis* and *O. claytoniana* have a higher genetic identity (0.373) than either of them does with *O. cinnamomea*. Based on calculation of Nei's (1972) genetic identities, cluster analysis of 19 populations resulted in the dendrogram shown in Fig. 2.

Table 4. Genetic variability among the populations of the three species of *Osmunda*. A: mean number of alleles per locus; P: proportion of loci polymorphic; H_{ob}: observed proportion of loci heterozygous; H_{exp}: expected proportion of loci heterozygous.

Species	A	P	H_{ob}	H_{exp}
O. regalis	1.48	0.277	0.071	0.121
O. cinnamomea	1.43	0.250	0.068	0.096
O. claytoniana	1.27	0.154	0.058	0.062

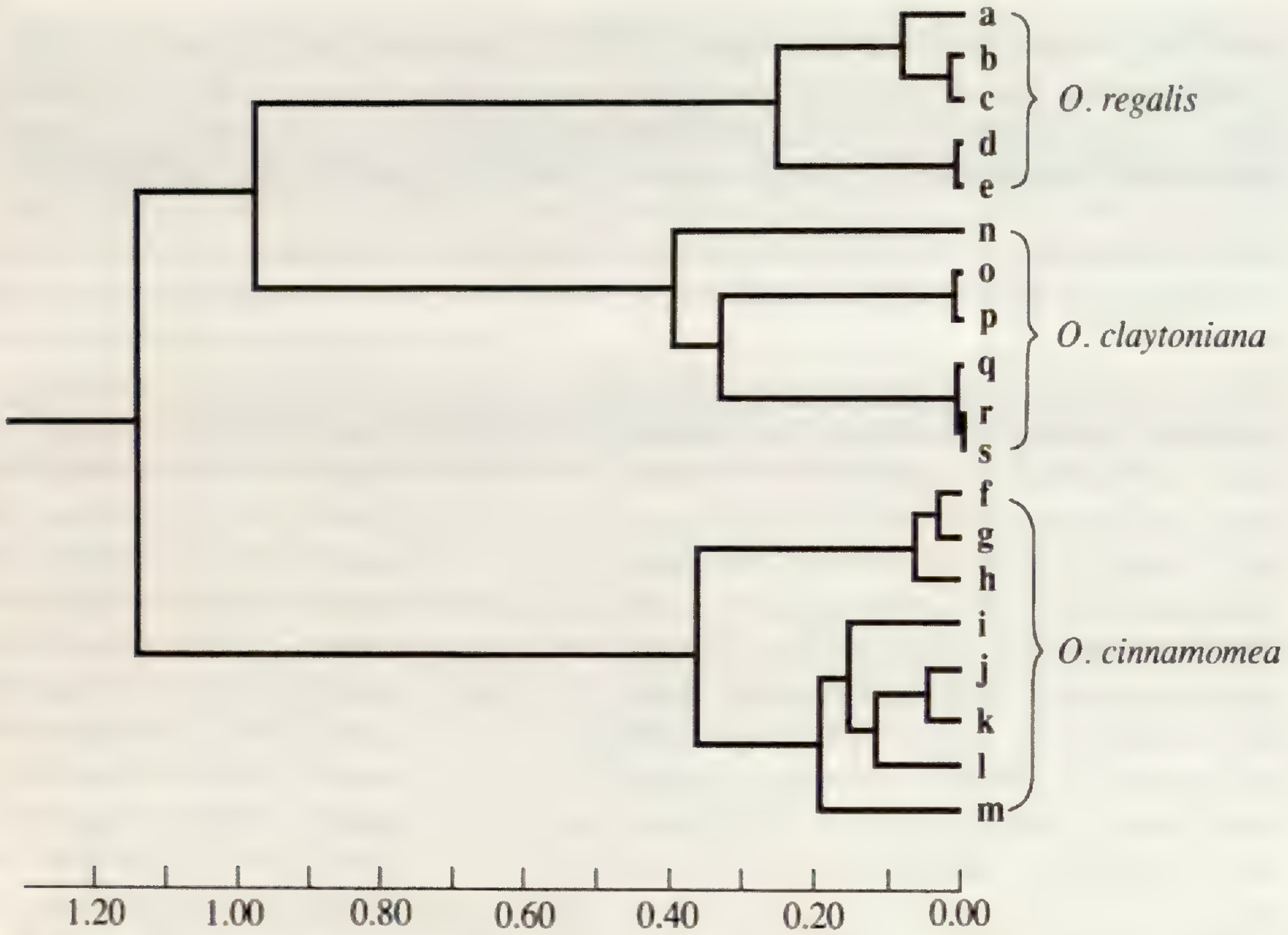


Fig. 2. Cluster analysis of 19 populations of *Osmunda* based on Nei's genetic distance. Letters correspond to populations listed in Table 1.

Discussion

Genetic Variability and Breeding System

The chromosome base number of *Osmunda* was hypothesized to be x=11 (Tatuno et al., 1966; Löve et al., 1977). All three *Osmunda* species we studied have a chromosome number of 2n=44 (Löve et al., 1977). Thus, they have been considered tetraploid. Because polyploid species are known to display complex banding patterns of isozymic expression, such as "fixed heterozygous," "unbalanced banded patterns," or "multiple banded patterns" (Crawford, 1990; Watano, 1991; Rabe & Haufler, 1992), *Osmunda* species might be expected to exhibit such banding patterns. However, only simple patterns were observed and the three species appeared to be genetic diploids.

Genetic variation of a species at different biological levels can be evaluated through proportion of loci polymorphic (P) and mean number of alleles per locus (A). The P and A are significantly dependent on differences among taxonomic status, life form, geographic range, and breeding systems of plants (Hamrick & Godt, 1990). For homosporous ferns, P ranged from 0.083 to 0.615 with a mean of 0.36, and A from 1.09 to 2.62 with a mean of 1.52 (Soltis & Soltis, 1990; Haufler et al., 1990). Comparison of our data on *Osmunda* species with available data indicated that the mean measures of genetic variation within populations (Table 4) fell within the range of variability, but had lower than the mean values of P and A. Previous allozymic analyses have concluded that greater intrapopulational genetic variation might be maintained in the plant species with outcrossing mating systems, non-isolated populations, and high levels of interpopulational gene flow (Hamrick et al., 1979; Soltis & Soltis, 1990). The genetic features of the three

Osmunda species might result from having inbreeding mating systems, isolated populations, and/or limited gene flow among populations.

Our allozymic analysis also indicated that 15 of 19 populations contained fewer heterozygotes than Hardy-Weinberg expectation (Table 3), and all three species had a deficiency of heterozygotes (Table 4). Furthermore, only three negative fixation index values (relative to nineteen positive values) with significant departures from zero were obtained (Table 7). These genetic conditions could result from small population sizes, disruptive selection, and/or frequent inbreeding. Because 80% of our populations contained an average of 31 individuals, the effect of population size should be minimal. It is likely that disruptive selection is not a significant factor and that inbreeding patterns within the populations are probably responsible for the deficiency of heterozygotes.

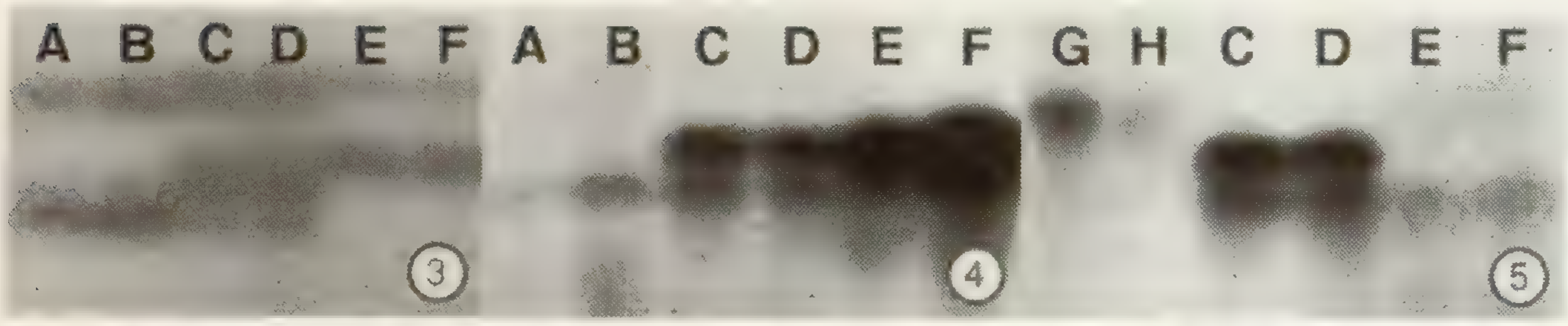
Table 5. Values of Nei's genetic identities (below the diagonal) and genetic distances (above the diagonal) among the 19 populations of *Osmunda*. The identifications of the 19 populations are given in Table 1.

Pop.	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s
a	**	0.054	0.080	0.286	0.299	0.929	0.900	0.985	0.886	0.912	0.877	0.870	0.809	1.127	0.871	0.836	1.026	1.064	1.057
b	0.947	**	0.009	0.230	0.235	0.972	0.971	1.026	0.920	0.939	0.908	0.907	0.963	1.137	0.826	0.774	0.929	0.965	0.985
c	0.923	0.991	**	0.220	0.226	0.979	1.030	1.034	0.922	0.955	0.918	0.902	0.968	1.271	0.813	0.759	0.908	0.948	0.938
d	0.751	0.795	0.803	**	0.001	1.061	1.029	1.110	1.126	1.069	1.059	1.144	1.143	1.366	1.192	1.121	1.067	1.060	1.090
e	0.741	0.791	0.798	0.999	**	1.114	1.095	1.161	1.142	1.063	1.056	1.173	1.150	1.136	1.220	1.146	1.017	0.999	1.036
f	0.394	0.378	0.376	0.346	0.328	**	0.028	0.021	0.297	0.286	0.370	0.263	0.390	1.242	1.246	1.160	1.201	1.232	1.231
g	0.407	0.379	0.357	0.357	0.336	0.972	**	0.047	0.376	0.335	0.382	0.338	0.467	1.168	1.317	1.226	1.270	1.290	1.299
h	0.373	0.358	0.356	0.330	0.313	0.979	0.954	**	0.352	0.346	0.439	0.326	0.452	1.335	1.230	1.146	1.340	1.369	1.372
i	0.412	0.399	0.398	0.324	0.319	0.743	0.687	0.703	**	0.127	0.208	0.171	0.288	1.156	1.448	1.348	0.881	0.908	0.907
j	0.402	0.391	0.385	0.343	0.345	0.751	0.715	0.706	0.881	**	0.038	0.071	0.169	1.176	1.507	1.400	1.142	1.152	1.166
k	0.416	0.403	0.399	0.348	0.348	0.691	0.682	0.645	0.812	0.963	**	0.134	0.236	1.166	1.486	1.385	1.127	1.145	1.154
l	0.419	0.404	0.406	0.319	0.309	0.769	0.713	0.722	0.843	0.931	0.875	**	0.109	1.149	1.426	1.327	1.093	1.140	1.126
m	0.425	0.382	0.380	0.319	0.317	0.677	0.627	0.636	0.750	0.845	0.790	0.897	**	1.215	1.525	1.507	1.170	1.191	1.199
n	0.324	0.321	0.281	0.255	0.268	0.289	0.311	0.263	0.315	0.309	0.312	0.317	0.297	**	0.525	0.524	0.288	0.287	0.283
o	0.419	0.438	0.444	0.304	0.295	0.288	0.268	0.292	0.235	0.222	0.227	0.240	0.218	0.592	**	0.008	0.291	0.291	0.286
p	0.433	0.461	0.468	0.326	0.318	0.313	0.293	0.318	0.260	0.247	0.250	0.265	0.222	0.592	0.992	**	0.288	0.291	0.284
q	0.358	0.395	0.403	0.344	0.362	0.300	0.281	0.262	0.414	0.319	0.324	0.335	0.310	0.750	0.748	0.750	**	0.003	0.000
r	0.345	0.381	0.391	0.336	0.368	0.292	0.275	0.254	0.403	0.316	0.318	0.320	0.304	0.751	0.746	0.746	0.997	**	0.000
s	0.347	0.373	0.391	0.336	0.355	0.292	0.273	0.254	0.404	0.312	0.315	0.324	0.301	0.754	0.751	0.753	1.000	1.000	**

The breeding system proposed from isozymic data contrasted with that developed through analysis of laboratory-grown gametophytes. Klekowski (1973) concluded that *Osmunda regalis* tended to be outcrossing based on high levels of genetic load. These discrepancies can be reconciled in a number of ways. Some of the populations investigated in this study were isolated and such isolation could lead to high levels of apparent homozygosity. Because polymorphism is low, even though fertilization occurred between individuals (outcrossing), the genotypes of the offspring for those loci would be homozygous. Consequently, more homozygotes would be discovered. Potentially also contributing to the different conclusions reached was that Klekowski experimented with gametophytes in vitro and inferred mechanisms of breeding in vivo. Results obtained under artificial conditions may not predict accurately what could happen in nature.

Table 6. Values of Nei's genetic identities (Below the diagonal) and genetic distances (above the diagonal) among the three species of *Osmunda*.

Species	*O. regalis*	*O. cinnamomea*	*O. claytoniana*
O. regalis	***	1.004	0.985
O. cinnamomea	0.366	***	1.256
O. claytoniana	0.373	0.285	***



Figs. 3–5. Photographs of representative gels testing hypotheses for the origin of the hybrid *O.* x*ruggii*. Top of photographs = anode, bottom of photographs = cathode. A and B = *O. regalis*. C and D = *O.* x*ruggii*. E and F = *O. claytoniana*. G and H = *O. cinnamomea*. Fig. 3. TPI. Cathodal band = locus 1 showing an identical allele in all species. Anodal bands = locus 2. Three-banded heterozygous pattern for this dimeric enzyme in individuals of *O.* x*ruggii* represents a combination of alleles from *O. regalis* and *O. claytoniana*. Figs. 4 and 5. LAP. Fig. 4. Two-banded heterozygous pattern for this monomeric enzyme in individuals of *O.* x*ruggii* represents a combination of alleles from *O. regalis* and *O. claytoniana*. Fig. 5. Heterozygous pattern in individuals of *O.* x*ruggii* do not contain marker alleles from *O. cinnamomea*.

Genetic Distance and Gene Flow

When the interpopulational genetic distances of the 19 populations were correlated with their geographic distributions (Fig. 1), in most cases genetic divergence was directly proportional to geographic distance. The three populations (**f, g** and **h**) of *O. cinnamomea,* for example, collected from the central states, exhibited low interpopulational genetic distance, while larger genetic distances were found between these three populations and the five populations (**i, j, k, l** and **m**) from northeastern states (Table 5). This correlation may infer a loss of gene flow between the two widely separated sets of populations. Even though spores are small enough to be carried far away from their parents by wind, they have to find a gametophytic "safe site" to survive. The fact that spores of *Osmunda* species are green and have a relatively short viability may also contribute to the loss of apparent gene flow over distance.

Table 7. Fixation indices for 19 populations of *Osmunda*

Pop.	GPI-1	PGM-1	PGM-2	TPI-2	LAP	HK	ALD	SkDH	MDH-1	MDH-2	MDH-3
a	0.240	---	0.746***	---	0.568**	0.204**	1.000***	0.780***	---	-0.010	---
b	-0.020	1.000	0.634***	---	0.075	0.678**	1.000	---	---	---	---
c	---	---	---	---	0.656***	-0.009	---	---	---	---	---
d	---	-0.030	---	---	---	0.157	1.000***	---	---	---	-1.000***
e	---	---	---	---	---	0.467	1.000	---	1.000	1.000	-1.000
f	---	0.307	-0.016	---	---	-0.433	---	-0.027	---	---	0.380
g	---	---	-0.051	---	-0.871***	-0.430	---	---	---	---	---
h	-0.067	1.000***	-0.022	1.000	---	0.540***	---	0.735***	---	---	---
i	-0.015	---	-0.048	---	---	-0.176	---	0.100	---	---	1.000***
j	-0.018	-0.098	-0.106	---	1.000***	0.159	---	---	---	---	0.289
k	-0.051	-0.091	-0.073	---	0.863***	0.727***	---	---	---	---	0.238
l	---	1.000***	-0.188	---	---	-0.021	---	---	---	---	0.099
m	---	---	---	---	---	1.000	---	---	---	---	---
n	-1.000***	---	---	---	---	-0.111	---	---	---	---	---
o	0.190	---	---	---	---	---	---	---	---	---	---
p	-0.143	---	---	---	---	---	---	---	---	---	---
q	0.033	-0.019	---	---	---	---	1.000***	---	0.135	---	---
r	-0.058	-0.023	-0.048	---	---	1.000***	---	---	-0.143	---	---
s	-0.-56	---	---	---	---	0.474	---	---	0.417	---	---

**: $p < 0.01$, ***: $p < 0.001$.

Phylogenetic Relationships

Analysis of electrophoretic data demonstrated that genetic distances (Nei, 1972) between conspecific populations were quite low (average = 0.233, range = 0.000 – 0.525), providing evidence that each species represented a genetically integrated unit. Even the widely separated populations of *O. regalis* from England shared a substantial

number of alleles with those from the US. In contrast, the three species of *Osmunda* were very distinct from each other (average = 1.103, range = 0.809 – 1.525). A dendrogram (Fig. 2) derived from genetic distance calculations suggested the following relationships among the three species: *O. regalis* may be more closely related to *O. claytoniana* than either of them is to *O. cinnamomea.* This isozymic phylogeny is consistent with the hypothesis of Faull (1901), Miller (1967, 1971) and Wagner et al. (1978). However, because of the large genetic distances among the three species and the small differences among the values of the genetic distances (Table 6), it is probable that isozymic data do not provide compelling evidence for falsifying or verifying any of the other hypotheses.

Table 8. Shared loci between *O.* ×*ruggii* and the three *Osmunda* species.

	O. regalis	*O. claytoniana*	*O. cinnamomea*
O. ×*ruggii*	12	12	6

Parentage of the Hybrid

Although the isozymic data could not provide convincing evidence for testing phylogenetic hypotheses, they were very valuable in studying hybrid origins. Because there are such large genetic distances between the species, each species has a large set of unique isozymic marker bands. These "isozymic fingerprints" can be used to follow the genomes during hybridization events.

The hybrid, *Osmunda* ×*ruggii*, was discovered for the first time in 1940 and was described as the natural cross of *O. claytoniana* and *O. regalis* (Tryon, 1940). Although its origin was proposed to involve *O. cinnamomea* (Wagner, 1974), Wagner et al. (1978) later reiterated that the parents of the hybrid were *O. claytoniana* and *O. regalis.* The isozymic data in this study demonstrated that *O. claytoniana* and *O.* ×*ruggii* share more loci than *O. cinnamomea* and *O.* ×*ruggii* (Table 8). Of the thirteen loci from the eight studied enzymes, twelve of them were shared by *O. claytoniana* and *O.* ×*ruggii*. The isozymic markers supported the hypothesis that the parents of the hybrid are *O. claytoniana* and *O. regalis* (Figs. 3 and 4) rather than *O. cinnamomea* and *O. regalis* (Fig. 5).

ACKNOWLEDGEMENTS

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Confirmation of a Hybrid *Isöetes* from New Jersey

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A number of interspecific hybrids in the genus *Isöetes* have recently been confirmed (Taylor & Luebke, 1988; Brunton & Taylor, 1990; Britton & Brunton, 1992). *Isöetes engelmannii* x *lacustris* was reported for the first time from New Jersey by Taylor in Montgomery & Fairbrothers (1992). The hybrid was initially identified from herbarium material; however, the authors agreed at the time that confirmation by chromosome counts was necessary. This paper confirms the identity of the hybrid by chromosome counts, provides details of the morphology and ecology, and designates a binomial.

Isöetes lacustris L. (*I. macrospora* Dur.) was discovered in Lake Wawayanda, Sussex County, New Jersey, by J. L. Edwards in 1937 (Chrysler & Edwards, 1947). This species is near its southern limit of geographical distribution in northern New Jersey, and was listed as endangered in the state by Fairbrothers & Hough (1973). The population has been observed by Montgomery since 1965.

Isöetes engelmannii A. Braun is common in lakes and ponds in northern New Jersey. It was first collected in Lake Wawayanda by J. L. Edwards and E. T. Wherry in 1938 (Taylor in Montgomery & Fairbrothers, 1992).

Taylor recognized the hybrid by its irregular, polymorphic megaspores (Wagner et al., 1986) during examination of specimens for the *New Jersey Ferns and Fern Allies.* The parents were deduced from the spore surface ornamentation and the presence of the possible parents in the same lake. *Isöetes engelmannii* x *lacustris* was first collected by J. L. Edwards and J. Dezer in 1937, and again by J. L. Edwards in 1938. Montgomery collected the hybrid with *I. lacustris* in 1965. All collections were labeled as *"Isöetes macrospora"*, the recognized name for North American *I. lacustris,* until Taylor's identification.

METHODS

A systematic survey of Lake Wawayanda was made in September 1992 and October 1993 to determine locations and sizes of populations of the two species and the relative abundance of individual hybrid plants. Notes were made on the substrate and associate species, and on species present at suitable water depth where *Isöetes* plants were not found. Selected plants were collected to provide material for morphological analysis, spores for size and surface ornamentation, and root tips for chromosome counts.

Megaspores from the suspected hybrid, *I. engelmannii,* and *I. lacustris* were air dried and placed in concavity slides. The greatest diameters, including ornamentation, were measured for 25 megaspores, using a compound microscope fitted with an ocular micrometer. Megaspore surfaces of the suspected hybrid and parents were examined using an Hitachi S-570 scanning electron microscope. Air dried megaspores were mounted on stubs using double coated 3M® No. 415 polyester transparent tape. Mounts were sputter coated with 20nm of gold palladium.

Microspores from the suspected hybrid, *I. engelmannii,* and *I. lacustris* were hydrated

in a water droplet on slides and mounted in Aqua-Mount® (Lerner Laboratories). Twenty-five microspores of each taxon were measured across their longest diameters as with megaspores.

Chromosome counts of the suspected hybrid were made from root tip squashes. Plants were floated in water for several days and roots were harvested as they grew from the rootstock. Roots up to 1 cm long with white apices were collected between 8 and 9 AM, pretreated in paradichlorobenzene for three hours, then fixed in a 3:1 solution of 95% ethanol and glacial acetic acid. Following the protocol used in Taylor & Luebke (1988), the apical 0.5 mm was removed and mounted in a drop of Hoyer's medium, macerated and squashed.

Results

We found that *Isöetes lacustris* can readily be distinguished from *I. engelmannii* by leaf morphology. Leaves of *I. lacustris* were thicker, more olive-green in color, and recurved or spirally twisted. Leaves of *I. engelmannii* were relatively thinner, bright green in color, and nearly straight (Fig. 1). Plants of the hybrid could be distinguished by their large size when mature, with many somewhat recurved leaves of olive-green color. The differences were confirmed by examination of megaspores from selected plants. Younger plants of the hybrid cannot be distinguished from *I. lacustris* (or confirmed until their abortive spores are present).

Megaspores of *I. lacustris* from Lake Wawayanda were large, 638–773 µm (mean=700, s.d. = ±34 µm) in diameter and uniformly globose. The ornamentation (Fig. 2) consists of irregular crests and branching to anastomosing ridges, with a distinctive papillate girdle on the distal side of the equatorial ridge (Taylor et al., 1993). Megaspores

Fig. 1. Plants of *Isöetes lacustris, I.* x*fairbrothersii* and *I. engelmannii.*

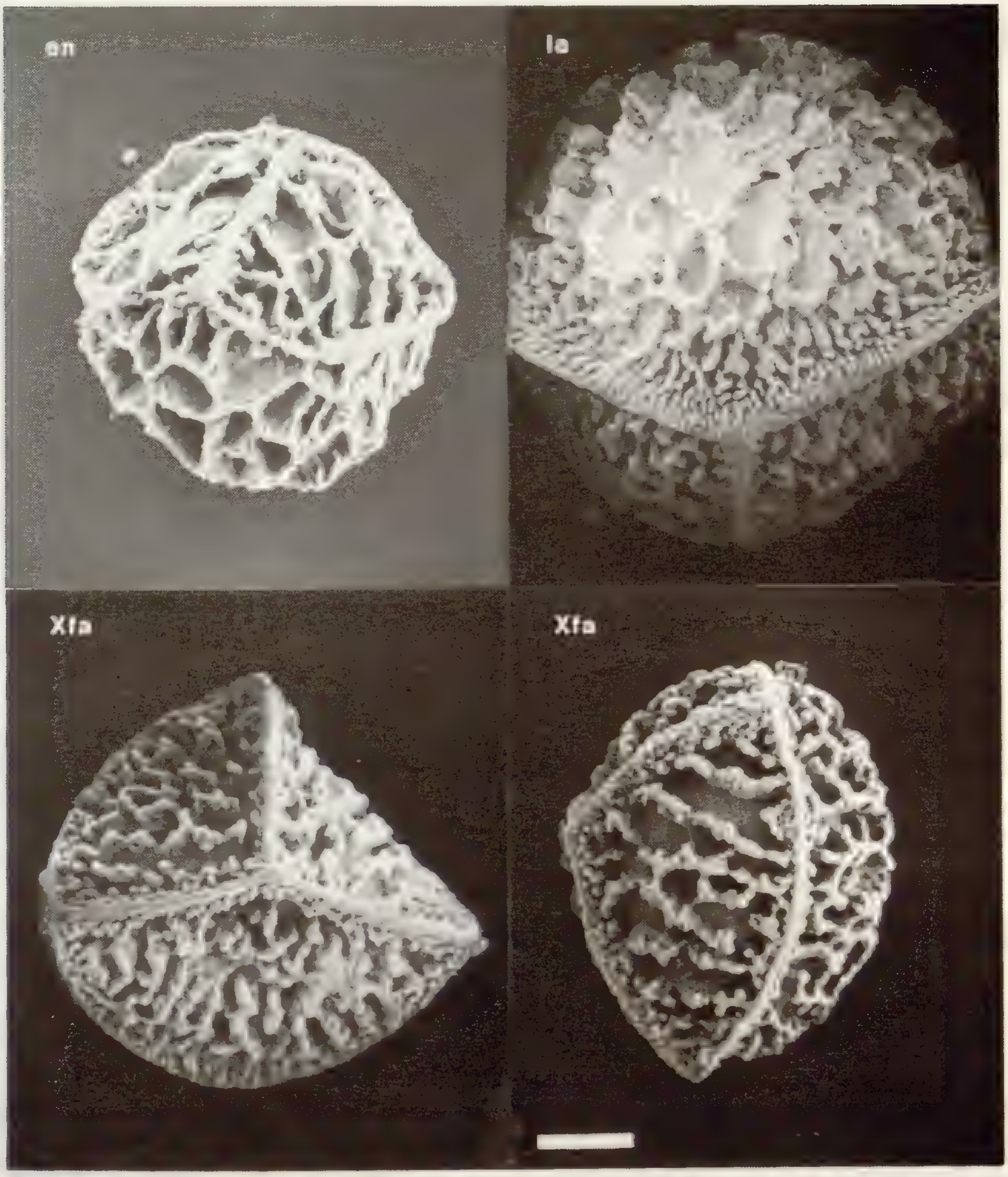

Fig. 2. Megasores of *Isöetes engelmannii* (en), *I. lacustris* (la), and *I.* x*fairbrothersii* (xfa). Bar = 120 μm.

of *I. engelmannii* were smaller, 470–556 μm (513±18 μm) in diameter, and uniformly globose. The ornamentation consists of regular reticulations on all faces with no papillate girdle. Megaspores of the hybrid were variable in size, 390–927 μm (532±104 μm) in diameter, and irregular in shape, with some very large and nearly spherical, others small and pyramidal, a few others dumbbell-shaped, and some represented by wedge-shaped fragments. The ornamentation consists of broken crests forming irregular reticulations, with a narrow papillate girdle on the distal side of the equatorial ridge and along the sides of the triradial ridges (Fig. 2). These characteristics can only be seen in mature megaspores; immature megaspores of both species and hybrid may be irregular in size and

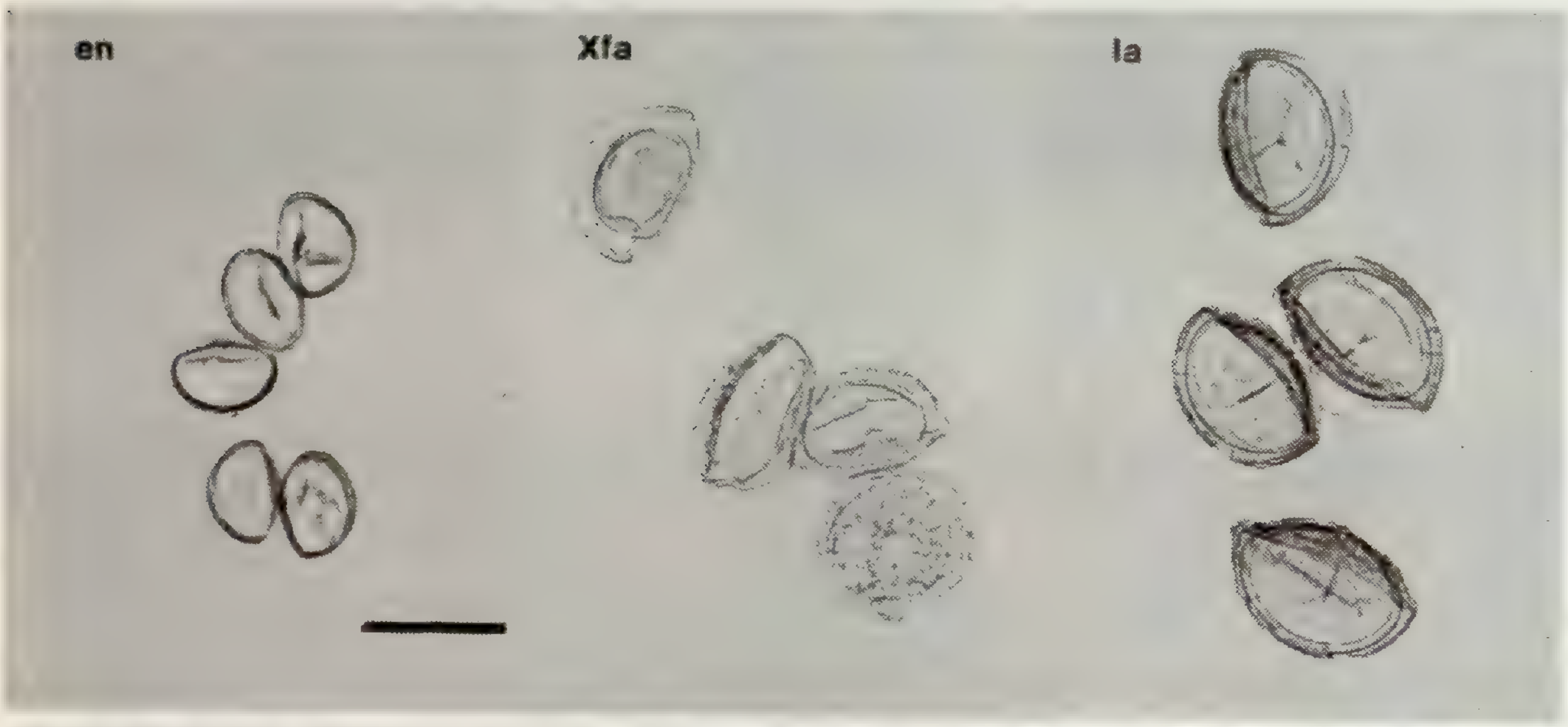

Fig. 3. Microspores of *Isöetes engelmannii* (en), *I. lacustris* (la), and *I.* x*fairbrothersii* (xfa). Bar = 36 µm.

shape. The plants used for this study, collected in September and October, had mature spores.

Microspores of *Isöetes engelmannii* were uniform in size and shape, 27–32 µm (30±1.5 µm) in longest dimension (Fig. 3). Microspores of *I. lacustris* were larger, 42–50 µm (46±2.1 µm), and also uniform in size and shape. Microspores of the hybrid varied from globose to reniform, and ranged from 35–45 µm (40±2.3 µm) in length. Most microspores of the hybrid appeared to contain little or no protoplasm, while microspores of *I. engelmannii* and *I. lacustris* are filled with protoplasm (Fig. 3).

Mitotic chromosome counts confirmed that the hybrid is hexaploid, 2n=66 (Fig. 4). This is the expected result of the cross of a diploid (*I. engelmannii,* 2n=22) and a decaploid (*I. lacustris,* 2n-=110).

Populations of *Isöetes* plants were found in "beds" around the shore of Lake Wawayanda and two small islands in the north end of the lake. Plants occurred in 0.2–2.0 m of water. The substrate consisted of sand or gravel; few plants were found in areas with deep organic substrate. Associated species included: *Eleocharis acicularis, Eriocaulon septangulare, Brassenia schreberi, Potamogeton crispus, P. robbinsii,* and *P. praelongus. Isöetes lacustris* tended to occur in deeper water (0.5–2.0 m) than *I. engelmannii* (0.2–1.5 m). The two species occurred together in a few locations, with *I. engelmannii* closer to shore and *I. lacustris* in deeper water. Hybrid plants were found as occasional individuals, mostly within *I. lacustris* populations.

No *Isöetes* plants were found in areas with a dense cover of *Myriophyllum spicatum* (Eurasian milfoil, an introduced species). The *Myriophyllum* was treated until 1989 with the herbicide Diquat and in 1992 with a limited application of Aquakleen (2, 4-D) (E. Zindel, personal communication); this appears not to have adversely affected the *Isöetes,* since both young and mature plants were found in abundance in many areas of the lake including areas where treatment was applied.

Four hybrids involving *I. lacustris* as one parent are known from North America (Britton & Brunton, 1992); only *I. engelmannii* x *lacustris* is known from New Jersey. Five hybrids are known involving *I. engelmannii* (Taylor, et al., 1993); three of these are known from New Jersey. The hybrid described here has not been reported outside New

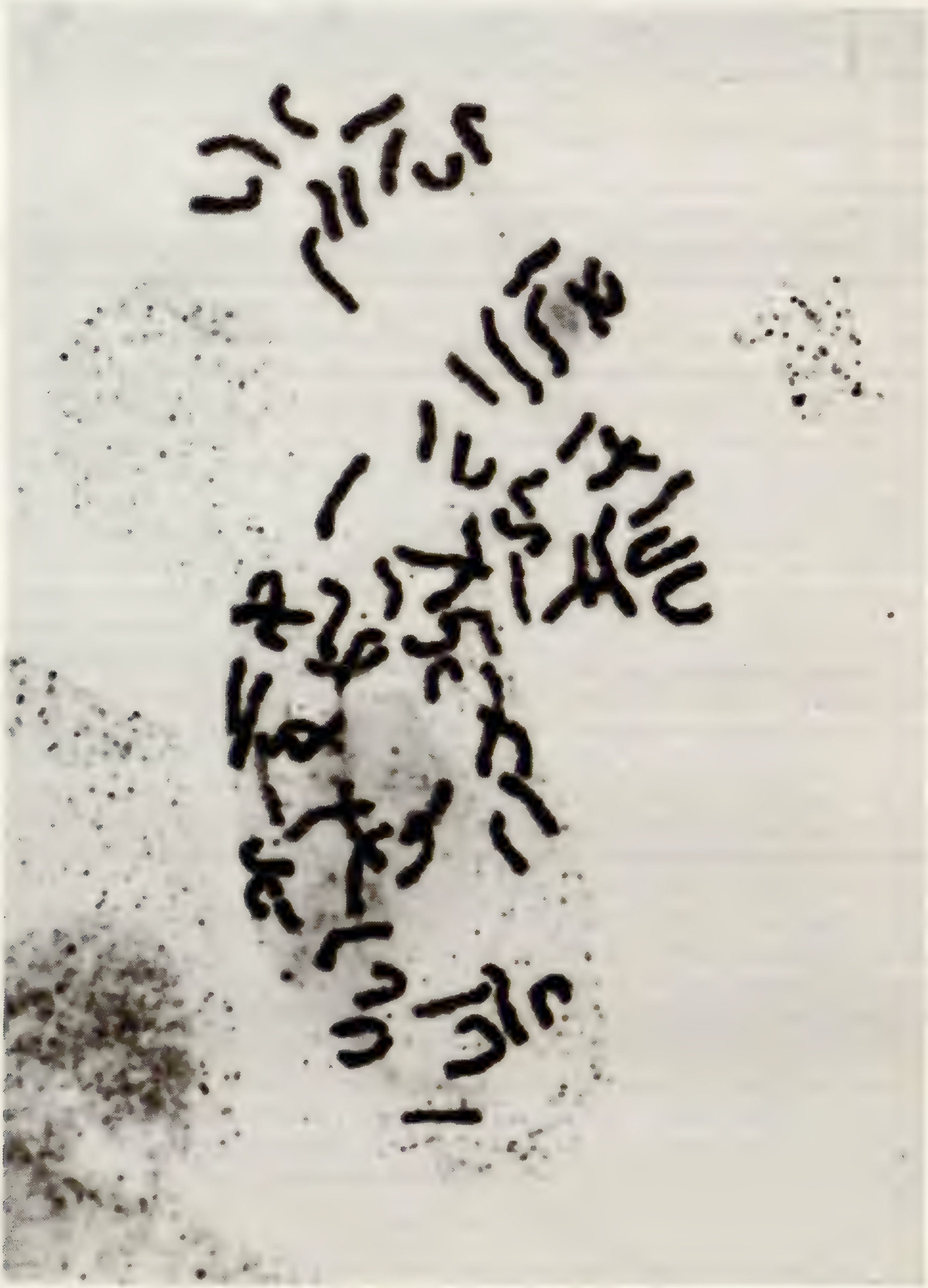

Fig. 4. Root tip squash of *Isöetes* x*fairbrothersii* showing 2n=66 chromosomes.

Jersey. The geographic range overlap of the parents is not extensive: northernmost New Jersey, New York, central and southern New England, and southern Ontario. Records of

Isöetes lacustris from Virginia and Tennessee, where *I. engelmannii* is more common, may represent a related taxon.

We propose to name this hybrid for Dr. David E. Fairbrothers, former Director of the Chrysler Herbarium at Rutgers University, for his extensive study of the flora of New Jersey.

Isöetes xfairbrothersii J. D. Montgomery and W. C. Taylor, hyb. nov.

Plantae inter *I. engelmannii* et *I. lacustris* interpositae. Megasporae forma et amplitudine variabiles. Chromosomatum numerus 2n=66.

HOLOTYPE: New Jersey, Sussex County, Lake Wawayanda, with *I. engelmannii* and *I. lacustris*. 3 October 1993. *J. . Montgomery & W. C. Taylor 93-325* (NY); isotype at CHRB. Other collections (all from Lake Wawayanda, N. J.): *J. L. Edwards s.n.*, 7 Aug. 1938; *J. D. Montgomery s.n.*, 2 Oct. 1965 (CHRB).

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A Summary of Chromosome Numbers in the Marsileaceae, with Counts for Additional Species of *Marsilea*

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Marsileaceae is a family of aquatic ferns containing three closely related genera: *Marsilea, Pilularia,* and *Regnellidium.* The genus *Marsilea* contains perhaps 50 species worldwide, while *Pilularia* contains six, and *Regnellidium* only one (Tryon & Tryon, 1982). All three genera have creeping, branched, and matted rhizomes bearing roots and upright leaves. They can be found growing rooted in aquatic environments or on land. The leaves of *Pilularia* are devoid of lamina, while those of *Regnellidium* bear two opposite pinnae, and those of *Marsilea* bear two pairs of pinnae (Bierhorst, 1971).

These plants are difficult to characterize as species because of the plasticity of their morphological features. Depending on the wetness of the environment, leaf size, shape, and hairiness, petiole length, and rhizome trichome abundance change, making consistent identification and classification difficult (Johnson, 1986). As a result, most taxonomic characters are found on the sporocarp; however, the only species of *Marsilea* which produce sporocarps abundantly in aquatic environments are *M. deflexa* and *M. berhautii* (Johnson, 1988). Because of these factors, chromosome data could provide useful taxonomic characters.

The chromosome numbers of the Marsileaceae have not been reliably reported for most species. A summary of the published reports of chromosome numbers for Marsileaceae is given in Table 1. Some of the early reports, including Marschall (1925) and Chrysler & Johnson (1939) were based on the thin section method, reducing their reliability, More accurate counts were done later using the squash method. Nearly all counts done later than the 1930s employed the squash method. The chromosome number for *Pilularia americana* A. Braun was recorded at $2n$=20 (Chopra, 1960). *Regnellidium diphyllum* Lindman was reported at $2n$=38, although 20% of the cells examined yielded $2n$=40 (Jain & Raghuvanshi, 1973). The two extra chromosomes are the first record of B chromosomes in pteridophytes. Their presence may be due to the fragmentation of some of the larger chromosomes (Tryon & Tryon, 1982). A reliable count of $2n$=40 for *Marsilea minuta* Linnaeus was reported by Mehra & Loyal (1958). In 1980, Bhardwaja recorded $2n$=40 for *M aegyptiaca* Willdenow (Bhardwaja, 1980).

The purpose of my study was to determine the chromosome numbers for *Marsilea mollis* Robinson & Fernald, *M. macropoda* Engelmann ex A. Braun in Kunze, *M. schelpiana* Launert, *M. vestita* Hooker & Greville, and *M. villosa* Kaulfuss. I also hoped to examine these counts in a phylogenetic context.

MATERIALS AND METHODS

Chromosome preparations were made for the following species: *M. schelpiana,* grown from plants received from Brooklyn Botanic Garden in 1989; *M. vestita,* grown from sporocarps of *Stanford s.n.* from Mason Co., Texas; *M. mollis,* grown from sporocarps of *Pringle s.n.* (F) from Oaxaca, Mexico; *M. villosa,* grown from sporocarps of *Wagner 84087* from Oahu, Hawaii; and *M. macropoda,* grown from plants received in 1989 from

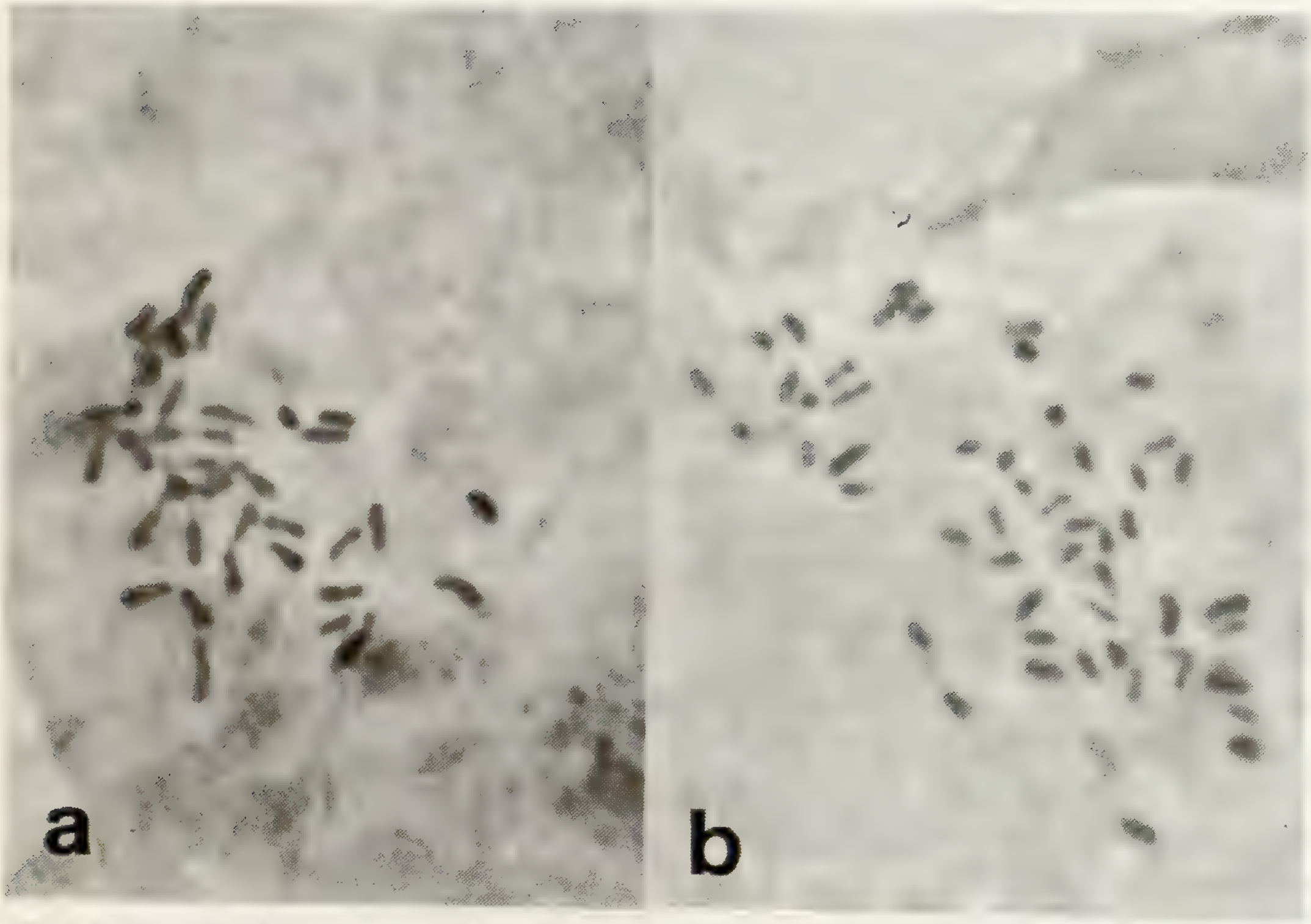

Fig. 1. Photographs of chromosomes of *Marsilea* species, X2000. a, *M. vestita*, 2*n*=40. b, *M. schelpiana*, 2*n*=50.

Fig. 2. Diagrams of chromosomes shown in photographs in Figure 1. a, *M. vestita*, 2*n*=40. b, *M. schelpiana*, 2*n*=50.

Georgia Southern College. The plants were grown in the greenhouse at Ohio Wesleyan University, and permanent voucher specimens can be found in the herbarium (OWU).

Table 1. Summary of published chromosome numbers for species of Marsileaceae.

Species	Chromosome Number	Author
M. drummondii[1]	2*n*=32	Strasburger (1907)
M. hirsuta[1]	2*n*=32	Strasburger (1907)
M. vestita[1]	2*n*=32	Strasburger (1907)
M. quadrifolia[1, 2]	2*n*=32	Strasburger (1907)
P. globulifera[1, 3]	2*n*=26	Litardière (1920)
M. quadrifolia[1, 3]	2*n*=32	Litardière (1920)
M. quadrifolia[1, 2, 4]	*n*=16	Marschall (1925)
M. quadrifolia[2, 3, 5]	2*n*=100–140	Gustafsson (1935)
M. drummondii[2, 3, 5]	2*n*=204–210	Gustafsson (1935)
R. diphyllum[1, 2]	*n*=16	Chrysler and Johnson (1939)
M. hirsuta[2, 6]	2*n*=64	Feller (1953)
M. diffusa	*n*=22	Boterberg (1956)
M. minuta[2, 3, 6]	*n*=20, 2*n*=40	Mehra and Loyal (1958)
triploid race of *M. minuta*, reproducing vegetatively[3, 6, 7, 8]	2*n*=60	Mehra and Loyal (1958)
P. americana A. Br.[2, 3, 6]	2*n*=20	Chopra (1960)
R. diphyllum[3, 9]	2*n*=38	Loyal (1961)
R. diphyllum[2, 3, 6, 10]	2*n*=38	Abraham, et al. (1962)
R. diphyllum	2*n*=38; in 20% of cells 2*n*=40	Jain and Raghuvanshi (1973)
M. aegyptiaca[3, 6, 11]	2*n*=40	Bhardwaja (1980)
M. Rajasthanensis[3, 6, 12]	2*n*=40	Bhardwaja (1980)

1. Chromosome number obtained using thin-section technique
2. Chromosomes derived from sporocarp material
3. Chromosomes derived from vegetative material
4. Source of species reported: Miss Ethel Thomas of Charleston, IL
5. Source of species reported: Dr. G. Haglund, Lund Botanical Garden
6. Chromosome number obtained using squash technique
7. Source of species reported: collection from Amritsar, India
8. Source of species reported: botanical Laboratory of University of Tennessee, Knoxville
9. Source of species reported: Dr. W. W Bloom of Valparaiso, IN and Prof. B. Sen, Vivekananda Laboratories, Almora
10. Source of species reported: Botanical Garden of Kerala University
11. Source of species reported: collection from Ajmer, India
12. *M. Rajasthanensis* = *M. aegyptiaca* (Launert 1968)

The soil balls were loosened in the pots to allow for aeration and increased growth of the root tips. The following morning, the terminal 5 mm root tips were removed and placed in a saturated solution of paradichlorobenzene (PDB) for four hours at room temperature (21°C). The root tips were removed from the PDB solution, blotted, placed in Farmer's Solution (1:3 glacial acetic acid: 100% ethanol), and then stored in the freezer until used.

The methods for chromosome staining and squashing are modified from those of Taylor & Luebke (1988). Root tips to be squashed were hydrolyzed for about ten minutes in 1 N HC1 at 60°C. The root tips were then rinsed using four changes of 95% ethanol for a period of at least 30 minutes. The alcohol was blotted off and the tips were placed in Wittman's hematoxylin (Wiley, 1971) for about 30 minutes and in glacial acetic acid for 3–5 minutes.

After the root tips were removed from the acid, approximately 1 mm of tip was cut and placed on a microscope slide. The segment was cut and mashed with a razor blade. A drop of Hoyer's medium was placed over the root fragments, and a cover slip was then applied. The slide was pressed between two pieces of wood using a C-clamp for about ten seconds. The resulting squash was examined for chromosomes at 400X. Slides that contained figures were sealed with clear nail polish to prevent drying. Chromosome figures were photographed at 2000X, using an Olympus phase-contrast microscope and Kodak™ TMAX100 Professional film for black and white prints.

Results

The chromosome numbers for *M. vestita* and *M. schelpiana* were determined to be $2n$=40 and $2n$=50, respectively (Fig. 1a, 1b). *Marsilea mollis, M. villosa,* and *M. macropoda* all had counts of $2n$=40, but photographs were not able to be taken due to the nonplanar nature of the squash. The following numbers of counts were taken for each species: *M. vestita,* 2, 1 photographable; *M. schelpiana,* 6, 2 photographable; *M. mollis,* 2, *M. macropoda,* 2. The chromosomes of all the species were oval to elliptical in shape, and those of *M. vestita* were longer than those of the other species.

Discussion

The counts of $2n$=40 for *M. mollis, M. villosa, M. macropoda,* and *M. vestita* concur with the counts reported since the 1950's that have used the squash technique. Earlier studies using the sectional technique have not been confirmed using modern methods.

It is significant to observe that *M. schelpiana* is definitely higher in chromosome number than the other observed species. It is unlikely that the higher number is due to polyploidy, since Kurth (1981) noted that *M. vestita* roots do not show the polyploidy exhibited by roots of some other ferns. From preliminary attempts at constructing a phylogenetic tree for species of *Marsilea,* it is known that *M. schelpiana* is located in a different group on the tree than the other species examined. Launert (1968) states that *M. schelpiana* is closely related to *M. capensis* A. Braun. Cytological investigation of *M. capensis* and other closely related species might provide some explanation of this apparent deviation. In conclusion, it may be stated that, excepting *M. schelpiana,* the base number of n=20 for *Marsilea* is supported by this study.

Acknowledgments

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A Technique for the Investigation of Genetic Variation in Filamentous Gametophytes of *Trichomanes* (Hymenophyllaceae)

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The gametophyte of most ferns is a small, thalloid, heart-shaped, short-lived structure. In some species of *Trichomanes* (Hymenophyllaceae), the gametophytes are filamentous and consist of a weft of inter-twining branched filaments. Such gametophytes are perennial and can reproduce asexually by means of gemmae. Independent colonies of gametophytes have been reported from North America (Farrar, 1967, 1992), Britain (Rumsey et al., 1990) and Central Europe (Vogel et al., 1993, Jérôme and Rasbach, 1994). These colonies of filamentous gametophytes can exist as very small patches (<10mm^2) or can cover huge areas (20m^2). Such a colony, whether large or small, may have arisen from a single spore, from several spores or, asexually, by establishment from one or more gemmae. A colony of filaments may represent either a single individual, or consist of many individuals interwoven. This presents a problem for the study of genetic variation of individuals in these independent colonies when using conventional methods, eg. allozyme analysis (Hamrick, 1990). Since the maximum length of a continuous individual filament that can be isolated from a colony is only about 60–100 cells, conventional allozyme analysis and DNA extraction methods are inapplicable. To extract DNA from a single gametophyte filament, ultrasonication was used directly to provide a crude DNA extract for DNA amplification. To establish the extraction and amplification techniques, gametophytes of four taxa of *Trichomanes* were compared using a large single-copy region of chloroplast DNA. Taberlet et al. (1991) have shown that the use of their universal primers for DNA amplification of the chloroplast intergenic spacers between the *trn*T and *trn*F genes is informative for a wide range of plant species.

MATERIALS & METHODS

Gametophytes of four species of *Trichomanes* are in culture at The Natural History Museum, London and the University of Manchester. The origins of the collections used in this investigation are as follows: 1) *T. bauerianum* Endl., Hawaiian Islands, U.S.A. coll. D.R. Farrar, 2) *T. intricatum* Farrar, U.S.A. coll. D.R. Farrar, 3a) *T. boschianum* Sturm, U.S.A. coll. D.R. Farrar, 3b) *T. boschianum* Sturm, U.S.A. coll. D.R. Farrar, 4a) *T. speciosum* Willd., Yorkshire, UK, coll. F. Rumsey, 4b) *T. speciosum* Willd., the Azores, Portugal, coll. A.C. Jermy.

DNA isolation. — Continuous branched pieces of about 100 cells of the filamentous gametophytes (about 5mm long) were isolated under a dissecting microscope. Each filament was washed in 0.5% Triton X-100 three times and rinsed three times in distilled water. A cleaned filament was placed in 50µL of 10x PCR buffer (Promega) consisting of

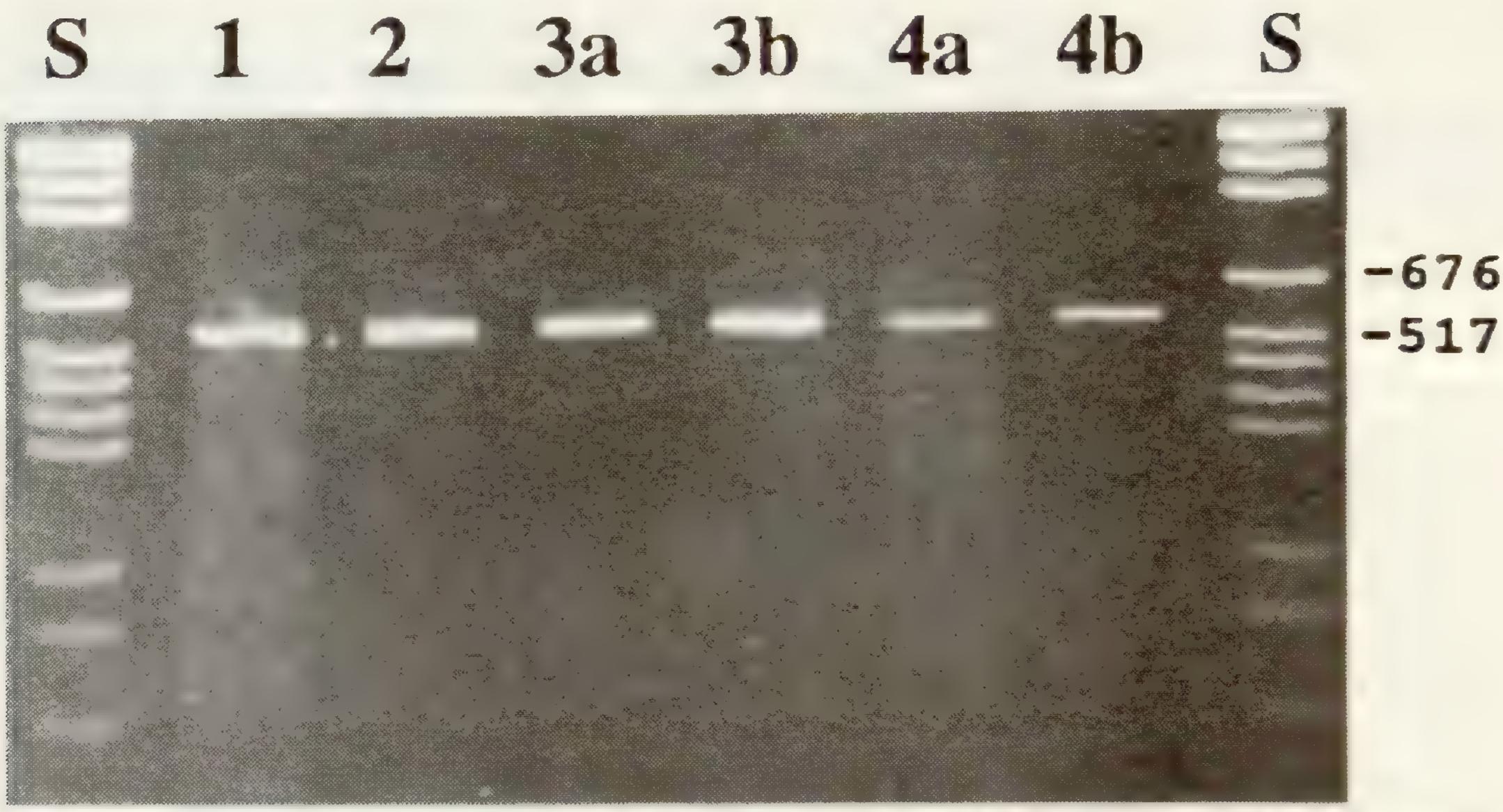


Fig. 1. Amplified product samples in the lanes are: 1: *T. bauerianum;* 2: *T. intricatum;* 3a: *T. boschianum;* 3b: *T. boschianum;* 4a: *T. speciosum;* 4b: *T. speciosum;* S: Standard DNA (pGEM Marker, Promega Co. Ltd.).

500mM KC1, 100 mM Tris-HC1 pH 9.0, 1% Triton X-100 in a 1.5mL Eppendorf tube. An Ultrasonic Cell Disruptor (Model VC 50, 50 Watt output. Sonics & Materials Inc. Danbury, CT U.S.A.) fitted with a tapered 3 mm diam. microtip disruptor horn was used to sonicate the sample for one second, 3 times, and the material was centrifuged at 16,000g to collect the solution from the tube wall. Microscopic examination confirmed that the treatment had ruptured the gametophyte cells. The sample was incubated with 2µL (20 mg/mL in distilled water) Proteinase K (Sigma) at 37°C in a water bath for 4 hours. Incubated samples were boiled for 15 minutes to deactivate any remaining enzyme. After dilution in distilled water to a ratio of 1 : 20, samples were used as DNA template for PCR with no further treatment.

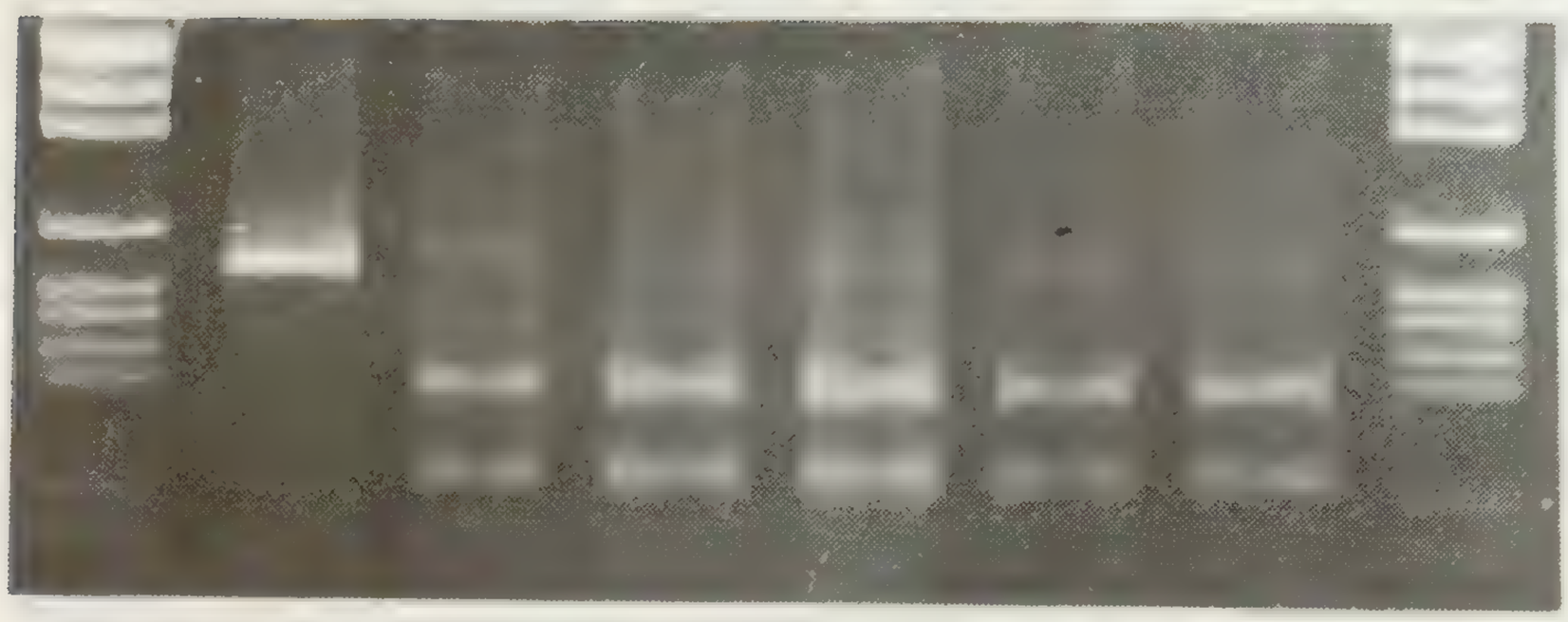
DNA Amplification. — The *trn*L (UGU) intron in the large single-copy region of chloroplast genome was amplified successfully from gametophyte population samples using primers C and D of Taberlet et al. (1991) Amplifications were performed in 100µL reaction volumes containing: 1.5mM $MgCl_2$, 200µM of each dNTP, 10 pmol of each primer, 3µL of diluted DNA template. The tube was overlaid with mineral oil and heated to 94°C for 5 minutes, then 4 units of Taq polymerase (Promega) was added. The amplifications were performed in a Techne PHC-2 DNA thermocycler programmed for 30 cycles at 94°C for 15 seconds, 50°C for 30 seconds and 72°C for 1 minute, using the maximum ramp rate between cycles and cooling to room temperature.

Restriction Digests and RFLP Analysis. — PCR products were digested with restriction endonucleases (Promega) in a 0.5mL Eppendorf tube at 37°C or 60°C in a water bath according to manufacturers instructions overnight. Restriction fragments were fractionated on a 2% agarose gel in 1x TBE buffer (Sambrook et al., 1989). Gels were stained with ethidium bromide, visualized by ultra violet-illumination and photographed.

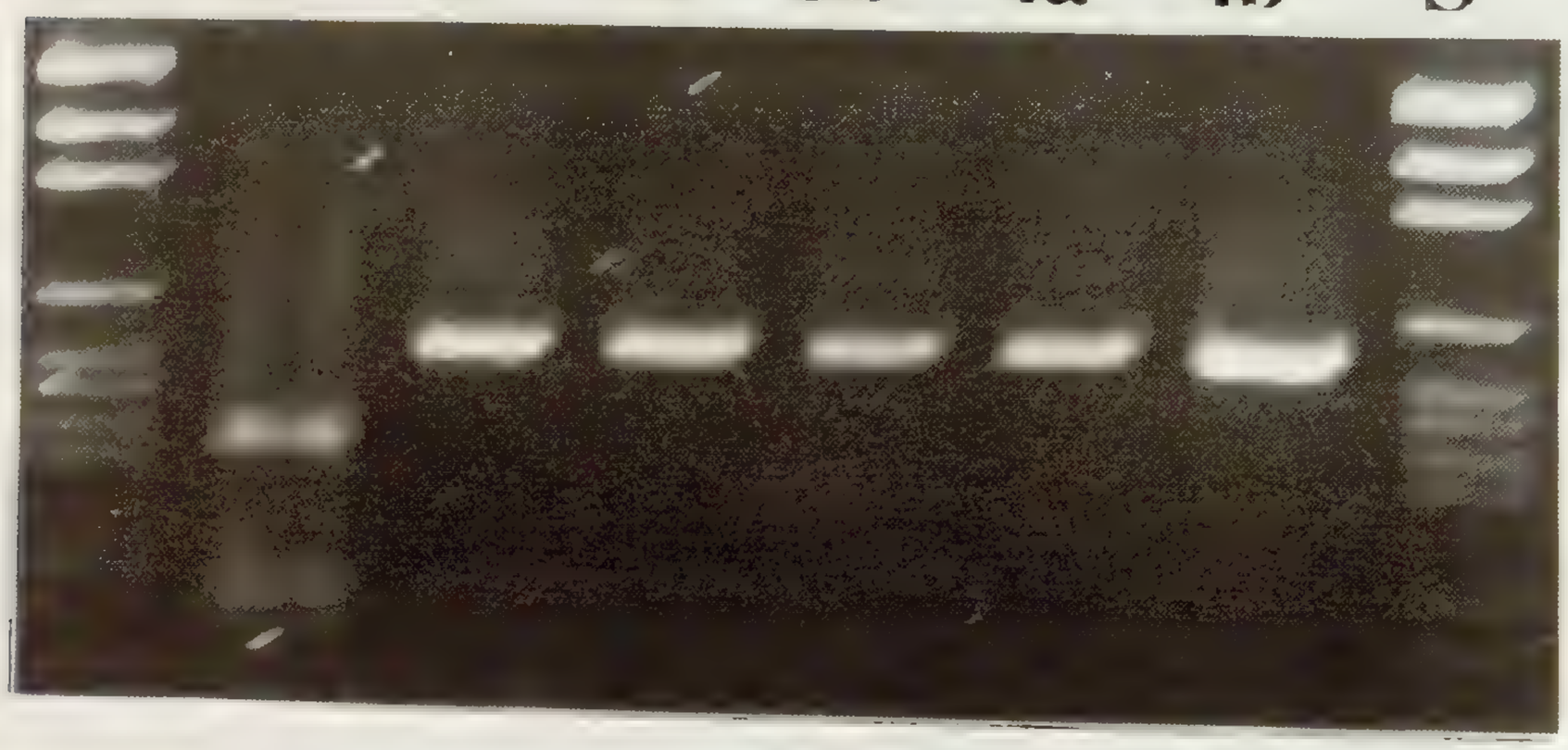
RESULTS

The amplification of the intron of the *trn*L gene generated 50ng/µl of PCR product of equal length for all taxa (about 530 base pairs, Fig. 1). When gametophyte samples were

A



B



C

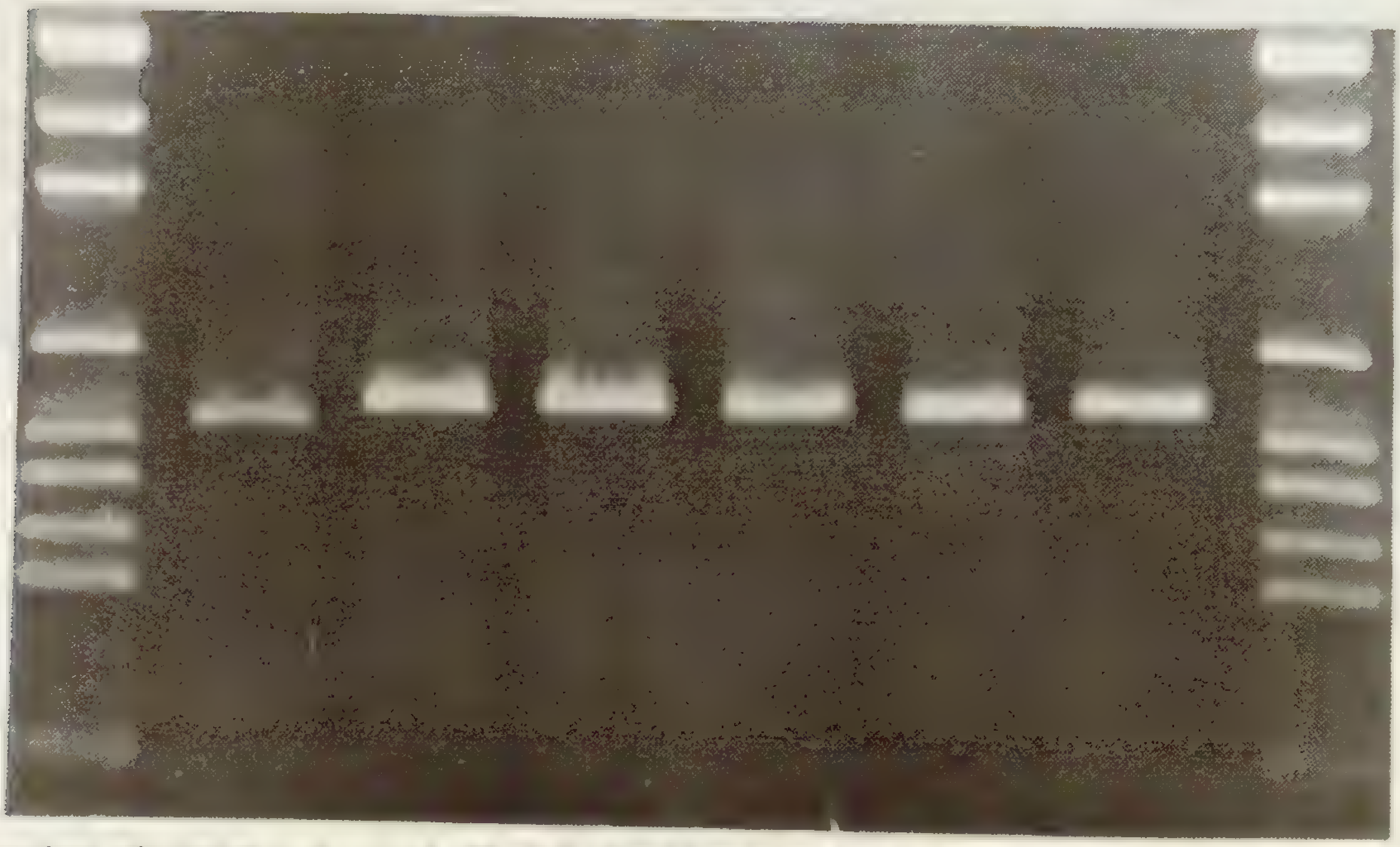


Fig. 2. Comparison of restriction digests. A: *Xho* I, B: *EcoR* I and C: *Msp* I. *T. intricatum* (lane2), *T. boschianum* (lanes 3a and 3b) and *T. Speciosum* (lanes 4a ;and 4b) had identical restriction patterns for all enzymes, but *T. bauerianum* (lane 1) showed consistent differences. S: Standard DNA (pGEM Marker, Promega Co. Ltd.).

ultrasonicated in distilled water the yields of PCR products were considerably lower (about 5–10 ng/µl) than using the 10X PCR buffer as a medium.

From a total of 27 restriction enzymes assessed, eight enzymes were informative, showing variation between the samples. These were *Ava*I, *Bgl*II, *Dra*I, *EcoR*I, *Hinc*II, *Msp*I, *Taq*I and *Xhol*I. Another 6 enzymes (*Hinf*I, *Mbo*I, *Rsa*I, *Sau*3AI, *Sty*I and *Tru*9I) have a restriction site on this *trn*L (UGU) intron DNA, but the results were identical for all four species. Another 13 enzymes (*Bam*HI, *cfo*I, *Hae*III, *Hind*III, *Kpn*I, *Not*I, *Pal*I (Pharmacia), *Pst*I, *Pvu*I, *Sac*I, *Sma*I and *Stu*I) had no restriction sites on this fragment. Digests performed with the enzymes *Xho* I, *EcoR* I, and *Msp* I are illustrated in Fig. 2.

Discussion

The method described yields amplifiable DNA from individual gametophytes. The absence of PCR product using the directly sonicated crude extractions as a DNA template may be due to the presence of cellular components such as phenolics (Tan and Thomson, 1989) that may damage nucleic acids or inhibit the PCR reaction unless diluted to negate their activity.

Although DNA isolation and amplification has been performed previously from crude extractions of single fungal spores (Lee & Taylor, 1990) and soil bacteria (Picard et al., 1992), the methods used were comparatively elaborate, involving either boiling the sample (in the case of individual spores) or sonication followed by microwave heating, thermal shocks and purification steps. The method reported here is simple and effective, and the extracted DNA can be used directly for PCR, or can be stored in a frozen state (-20°C) in excess of one year. The RFLP analysis of PCR product obtained with our new method indicates some variation between the samples, but with such a small sample size no significance can be attached to the results. This technique should also be appropriate for investigation of genetic diversity and phylogeny of other small or filamentous plants such as algae or bryophytes.

Acknowledgements

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Shorter Notes

Leaf flavonoids in the Malaysian *Coryphopteris gymnopoda* (Baker) Holtt. (Thelypteridaceae). — As part of our continuing studies of the flavonoid analysis (Umi Kalsom, Pertanika 21:149–152, 1991), we report on the flavonoids of *Coryphopteris gymnopoda*. The specimens were collected at the Cameron Highlands, Pahang, West Malaysia. Voucher specimens were deposited at the herbarium of the Department of Biology, Universiti Pertanian Malaysia. The pinnae were extracted with 70% ethanol and run by two dimensional paper chromatography in BAW and 15% HOAc on Whatman No. 1 paper. Three flavonoid glycosides were isolated and purified from 70% ethanol leaf extracts on Whatman No. 3 paper using standard solvents. Kaempferol-7-glucoside, genkwanin-4'-glucoside and quercetin-3-digalactoside (Table 1) were identified by standard procedures and are reported for the first time from this fern. Flavonoids are valuable compounds that have been used to clarify taxonomic relationships between the species of the Thelypteridaceae (Umi Kalsom, Biochemical Systematic and Ecology 21:743,1993). The flavonoid pattern thus revealed in *Coryphopteris gymnopoda* is generally an expectable one, in view of its taxonomic position in the Thelypteridaceae.

Table 1: Flavonoid identification in the pinnae of *Coryphopteris gymnopoda*

Flavonoid glycosides	colours in Uv/Uv+NH_3	Rfs in BAW[1]	15%HOAc	UV spectrum 80% MeOH	+NaOAc	+H_3BO_3	NaOH
Genkwanin 4'-*O*-glucoside	Dk/Y	41	53	334, 272	386, 305.5 281.5	355, 311 281.5	401(dec.), 334 382.5
Kaempferol 7-*O*-glucoside	Y/Y	60	28	371, 269	379, 274	365, 268.5	395.5, 277
Quercetin 3-*O*-digalactoside	Dk/Y	39	55	356, 256.5	379, 271	377.5, 261	387, 270

[1](BAW: 4:1:5, top layer)

The author thanks the Universiti Pertanian Malaysia for the financial support. — Umi Kalsom Yusuf, Department of Biology, Faculty of Science and Environmental Studies, Universiti Pertanian Malaysia, 43400, Serdang, Malaysia.

The Thelypteridaceae in Idaho. — Recent treatment of the Thelypteridaceae in Volume 2 of the *Flora of North America* (Flora of North America Editorial Committee, eds., 1993, New York: Oxford University Press) omitted mention of the taxon in Idaho. Two species in the family, *Phegopteris connectilis* and *Thelypteris nevadensis,* have been documented from the state during the last two decades and the objective of this note is to chronicle that fact.

Phegopteris connectilis (Michaux) Watt [*Thelypteris phegopteris* (L.) Slosson] is a circumboreal species whose distribution in western North America was thought to include Alaska, south to northern Oregon and northwestern Montana *(Flora of North America,* Vol. 2). Since 1987, intensive floristic inventories of northern Idaho have been conducted by the Conservation Data Center and funded by the U.S. Forest Service. One result of these surveys was the discovery of nine populations of *P. connectilis* in two areas of the Idaho panhandle. It was first discovered in Boundary County in 1990, at Robinson Lake in the Kootenai River drainage, Lat. 48° 58' 12" N, Long. 116° 13' W (*Lorain 2102* ID, *Bursik 1809* ID). All subsequent populations have been discovered in the Priest River Valley, Boundary and Bonner counties, ca. 56 km west of Robinson Lake, between Lat. 48° 34' 16" and 48° 53' N, and Long. 116° 52' 18" and 116° 58' 46" W (*Bursik 1837, 1863, 2102, 2107, 2120, 2121, 2495* ID). The populations are small and localized, with

all except one inhabiting the Kaniksu National Forest. It occurs in the understory of mature and old-growth *Thuja plicata* and *Tsuga heterophylla* forests between 756 m and 1097 m. The substrates are moist to wet, loamy soil covered with deep duff. Associated species include *Athyrium felix-femina, Tiarella trifoliata, Lycopodium annotinum, Cornus canadensis, Gymnocarpium dryopteris, Oplopanax horridum,* and *Circaea alpina.* A recent search of regional herbaria for taxa considered rare in Idaho did not locate other specimens of *Phegopteris connectilis* from Idaho (unpublished data on file at the Conservation Data Center, Boise, ID). The Idaho populations lie 29 km northwest of the nearest population in Montana (unpublished data on file at the Montana Natural Heritage Program, Helena, MT) and approximately 245 km south of the nearest populations in British Columbia (Adolf Ceska, Royal British Columbia Museum, Victoria, 1993, personal communication).

Thelypteris nevadensis (Baker) Clute ex C.V. Morton was thought to occur largely west of the Cascade-Sierran axis from southern British Columbia to northern California (*Flora of North America,* Vol. 2). In 1971, Fred Johnson and Bob Steele, from the University of Idaho, discovered a population in the Isabella Creek drainage, a major tributary of the North Fork of the Clearwater River in north-central Idaho, Lat. 46° 54' 40" N, Long. 115° 34' 34" W [*Johnson and Steele 7156* ID, *7157* ID (determined by D. Wagner, University of Oregon, 1972), *Lorain 1983* ID]. During intensive surveys for rare plants in the Clearwater River basin in 1993, another population was discovered two miles away (*Kingsbury s.n.* ID). Similar to *Phegopteris connectilis,* the two Idaho populations are small and localized and both are managed by the Clearwater National Forest. *T. nevadensis* occurs on the moist banks of small creeks in *Thuja plicata, Abies grandis,* and *Alnus rubra* stands between 940 m and 1025 m. It occurs on both mineral and organic substrates with *Cornus canadensis, Boykinia major, Senecio triangularis, Equisetum sylvaticum, Asarum caudatum, Adiantum aleuticum,* and *Rubus parviflorus.* Based on the search of regional herbaria mentioned above, these populations appear to be the first documented from Idaho, and represent a disjunction of at least 500 km east of the nearest populations in the Cascade Range. — ROBERT K. MOSELEY, CHRISTINE LORAIN-EBRAHIMI, ROBERT J. BURSIK, JUANITA LICHTHARDT, Conservation Data Center, Idaho Department of Fish and Game, Boise, Idaho, 83707.

Ferns on Fossiliferous Eocene Formations in Maryland. — The Eocene strata of the coastal plain in Maryland and adjoining states extends in a nearly continuous belt of varying width south-southwestward from New Castle Co. in northern Delaware as far as the Nottoway River in southeastern Virginia. In Maryland east of Chesapeake Bay, the strata can be seen in Kent Co. and Queen Annes Co. on the Delmarva Peninsula and on the northern portion of Kent Island. West of Chesapeake Bay, the strata are mostly exposed along creeks from Anne Arundel Co. through Prince Georges Co. to Charles Co. at the Potomac River. Good exposures of fossiliferous rock occur along all the leading stream channels, with some broad outcrops at the surface. The strata are divided into two formations, the Aquia Formation, lying to the northwest, and the Nanjemoy Formation, lying to the southeast but absent from the Delmarva Peninsula and Delaware. One of the best exposures of Eocene strata occurs along the north side of the Chester River about two miles south of Chestertown, with outcrops exposed up to about ten feet high at low tide. Large blocks have cleaved from the cliff face, perhaps because of root growth of large Chestnut Oak trees near the edge of the cliff. Interesting ferns, accessible most readily by

boat, live on the cliffs and bluffs. Several years ago, the junior author explored this area while fishing on the river.

Collections from the cliffs include *Pellaea atropurpurea* (*Hadaway,* 2 Jul 1989, juveniles; *Reed 127485,* 7 Aug 1989), a first record for this fern on the coastal plain. A dense stand of *P. atropurpurea* was found on the top and sides of a cleaved block standing in the water, along with a few plants of *Arabis lyrata.* On the cliff face *Camptosorus rhizophyllus* grew in dense, flattened mats, along with several much larger plants of x*Asplenosorus ebenoides.* These are also first records for the coastal plan of the Delmarva Peninsula. At the rim of the cliff were mature plants of *Asplenium platyneuron,* one parent of x*Asplenosorus ebenoides. Polypodium virginianum* grew at the edge of the cliff, extended several feet back to the bases of the oak trees (with a few specimens of *Cunila organoides),* and sometimes advanced up their trunks. This species is rare on the Delmarva Peninsula; it is said to occur near Salisbury, Md.

On two occasions, the senior author also studied the adjacent area, for about a mile northwest of the cliff site, along MD-664 near the golf course. The soils are derived from the Eocene strata and include roadside slopes, seepages, a pond, and a few outcrops. He found *Athyrium filix-femina, Botrychium dissectum* var. *obliquum, Dennstaedtia punctilobula, Onoclea sensibilis, Osmunda regalis* var. *spectabliis, Polystichum acrostichoides, Thelypteris hexagonoptera,* and *T. palustris.* More than 200 specimens of flowering plants and ferns were collected by the senior author and are deposited in the Reed Herbarium. In addition, *Lygodium palmatum* was found in a wooded ravine on MD-664, off MD-289, near the river closer to Chestertown (*Hadaway* in 1988). — CLYDE F. REED, Reed Library and Herbarium, 1222 Main St., Darlington, MD 21034-1416, and ERIC C. HADAWAY, Route 664, Chestertown, MD 21620.

Large Population of *Pilularia americana* A. Br. In Arkansas — Populations of American Quillwort are known from Arkansas counties (Washington, Logan, Conway, and Garland). On July 1, 1993, I found *Pilularia* growing in rocky, shallow waters on the edge of Engineers Lake on Camp Joe T. Robinson (Arkansas Army National Guard) in Pulaski County, a county record. When I returned on October 6, after the lake had undergone a drawdown, I noticed a large area (approximately 150 sq. m), usually under approximately 8–16 cm of water, that was covered by vegetation that apeared to be a bluegrass lawn. This expanse extended about 6 m from the original shoreline at a gradual slope and stretched approximately 25 m along the shore. The population was quite dense and a relatively pure stand, with few invading species. Plants were about 3–4 cm tall with sporocarps scattered along rhizomes. Since the drawdown had occurred only 3 weeks earlier, the population probably thrived in the shallow water where it grew unnoticed; previous drawdowns had occurred in 1975 and 1988. This seldom seen, graminoid pteridophyte may be more common than herbarium records indicate. The adjacent shoreline area of Engineers Lake is almost entirely covered with herbaceous growth. Genera at the shoreline include *Juncus, Elcocharis, Carex, Cyperis, Hydrolea, Polygonum,* and *Rotala.* No trees are in the immediate vicinity. A voucher specimen was deposited (*Getz 427* UCA det. D.E. Culwell). — DONALD E. CULWELL, Department of Biology, University of Central Arkansas, Conway, AR 72032.

Pteridophyte Protein Extraction Procedures in Utilizing SDS-PAGE — Starch gel is in widespread use as an electrophoretic medium for the extraction of proteins largely due to safety factors. SDS-PAGE gels can be prepared in concentrations of 3–20% in sieving/resolving and gradient preparations. These gels can be purchased or prepared in the lab using 0.4% SDS added to the standard acrylamide solutions of Davis (Ann N.Y. Acad. Sci. 121: 404–427, 1964), or other acrylamide preparations. The utilization of gradient gels was found to produce more uniform separation and more distinct bands. We found that the protein bands were not as distinct in the SDS gels due to a lack of proteins in the extract. Unsatisfactory results were obtained utilizing extraction procedures outlined in the literature.

With modifications of one of the methods outlined by Soltis et al. (Amer. Fern J. 73:9–27, 1983), we were able to extract a usable complement of proteins. The procedure was carried out at 4°C in chilled mortars until centrifugation. The material to be used is thoroughly washed in glass distilled water and blotted dry. The extraction buffer is prepared using 0.1 M Hepes, pH 7 solution, 2% mercaptoethanol, 5% metabissulfite, and 1% SDS. The grinding mixture consisted of 0.2g tissue and 0.2g caffeine added to 1 ml of the extraction buffer. Grinding is carried out for five minutes in a cold mortar. Additional liquid may be required at this point. A small amount of the SDS running buffer consisting of 0.030% TRIS, 0.014% glycine, and 0.010% SDS was used if additional liquid was necessary. Periodic grinding was continued for twenty-five minutes allowing time for the protein extraction. The mixture is centrifuged at 10,000rpm for five minutes. The pellet is discarded, and the supernatant, cast in sieving gel or suspended in sucrose, is used as the sample. The most important finding at this time is that the addition of 1% SDS in the grinding medium enhanced the extraction, separation and resolution of the protein bands. We have made successful extractions from *Lycopodiella sp., Pseudolycopodiella, Selaginella apoda* complex, *Selaginella arenicola,* and *Isoëtes melanopoda.* — JAMES R. BRAY and DENNIS W. MCMASTERS, Department of Biology, Henderson State University, Arkadelphia, AR 71999-0001.

Review

Polypodiaceae — Dryopteridoideae – Phytsematieae by Robert G. Stolze, Leticia Pacheco & Benjamin Øllgaard, in G. Harling & L. Andersson, eds., Flora of Ecuador 49. 1994. Available from the Council for Nordic Publications in Botany, Botanical Museum, Gothersgade 130, DK-1353 Copenhagen K., Denmark. ISNB 87-88702-70-7.

This latest pteridophyte fascicle of the Flora of Ecuador extends the useful treatments of the Lycopodiaceae, Dicksoniaceae, Lophosoriaceae, Metaxyaceae, Cyatheaceae, Thelypteridaceae, and Aspleniaceae that were published previously in this series. The present fascicle contains the large genus *Diplazium* (55 spp.) and the other small genera of Woodsiaceae allied to it. Among the genera of Andean ferns, *Asplenium, Diplazium, Elaphoglossum,* and *Thelypteris* are the largest and most difficult taxonomically. Of these genera, *Diplazium* alone has fronds of most species several times larger than herbarium sheets, and so most specimens available to taxonomists are mere fragments. In addition, pinna morphology is often quite variable along the rachis. This adds greatly to the difficulty of understanding variation within fronds and, consequently, within species; it is easy to name small and large pinnae as different species. As he did previously in *Asplenium,* Stolze has produced a highly useful treatment, including a workable key,

species descriptions, distributions, notes, and specimens cited. Of particular note is the care with which he evaluated the scattered, inconsistently labeled, and usually fragmentary collections made by Father Luis Sodiro in Ecuador, many of which are types or syntypes of Sodiro's multitudinous species. Most botanists, myself included, have made too many facile assumptions about the status of these specimens. By virtue of his seeing more Sodiro material from more and different herbaria than anyone else, Stolze's evaluations of these specimens' status is as accurate as is possible. In any long and complex treatment, one can find a few things to object to. In my opinion, *D. striatastrum* is a valid species present in Ecuador, not a synonym of *D. striatum,* and the correct name for *D. prominulum* Maxon is *D. subsilvaticum* Christ in Pitt. That said, I have only praise for this treatment and congratulations for its authors. — DAVID B. LELLINGER, Dept. of Botany MRC-166, Smithsonian Institution, Washington, DC 20560.

Announcement

Beginning with the next issue (volume 85, number 1), the American Fern Journal will pass into the hands of a new editor: George Yatskievych, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166-0299, USA. Authors submitting new manuscripts should send them to the above address.

Referees for 1994

I thank the Associate Editors and the referees listed below for their assistance in the review process. Their evaluations of manuscripts submitted to the American Fern Journal have aided the authors, made my job easier, and contributed to the quality of our journal — JAMES H. PECK.

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